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The risk of escape ovulation under treatment with low-dose combined oral contraceptives

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ACADEMIC DISSERTATION

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by the Roman numbers I to IV:

I Elomaa K, Rolland R, Brosens I, Moorrees M, Deprest J, Tuominen J, Lähteenmäki P. Omitting the first oral contraceptive pills of the cycle does not automatically lead to ovulation. Am J Obstet Gynecol 1998;179:41-6.

II Elomaa K, Lähteenmäki P. Ovulatory potential of preovulatory sized follicles during oral contraceptive treatment. Contraception 1999;60:275-9.

III Elomaa K, Ranta S, Tuominen J, Lähteenmäki P. The possible role of enterohepatic cycling on bioavailability of norethisterone and gestodene in women using combined oral contraceptives. Contraception 2001;63:13-8.

IV Elomaa K, Ranta S, Tuominen J, Lähteenmäki P. Charcoal treatment and risk of escape ovulation in oral contraceptive users. Hum Reprod 2001;16:76-81.

ABBREVIATIONS

$AUC_{0\text{-}24h}$	24-hour area under the curve
BMI	body mass index
C_{max}	maximum concentration
CV	coefficient of variation
CYP	cytochromal P450 (enzyme)
DSG	desogestrel
EE_2	ethinylestradiol
EHC	enterohepatic circulation
FSH	follicle-stimulating hormone
GEST	gestodene
GnRH	gonadotropin-releasing hormone
HCG	human chorionic gonadotropin
IFMA	immunofluorometric assay
IRMA	immunoradiometric assay
LH	luteinizing hormone
LNG	levonorgestrel
LUF	luteinized unruptured follicle
LYN	lynestrenol
MD	monophasic desogestrel
MG	monophasic gestodene
NET	norethisterone
NET Ac	norethisterone acetate
NGM	norgestimate
OC	(combined) oral contraceptive
RIA	radioimmunoassay
SD	standard deviation
SHBG	sex hormone-binding globulin
TG	triphasic gestodene
t_{max}	time to maximum concentration
US	ultrasonography

ABSTRACT

The risk of escape ovulation was investigated in women using a variety of low-dose combined oral contraceptives, under challenge in simulated conditions.

Noncompliance was simulated by either lengthening the standard 7-day pill-free period by three days or until a follicle of predetermined size of 16 mm in diameter developed. Despite complete pituitary recovery by the end of the first pill-free week in most women, and variable degrees of ovarian activity, no ovulation was detected in 98 artificial cycles during or after the 10-day pill-free period. However, follicular growth of ≥13 mm occurred in 53% of women and follicles became most developed in women using a monophasic desogestrel (DSG) pill containing 20 μg ethinylestradiol (EE_2), compared with women on monophasic gestodene (GEST) ($p=0.015$) or triphasic GEST pills with no less than 30 μg of EE_2 per day. It is concluded that escape ovulation is rare in women using GEST and DSG preparations containing as little as 20 μg of EE_2 per day, when not more than the first three pills are omitted. Extending the pill-free period to 14 days, to allow a follicle to grow to 16 mm in diameter, on the other hand, led to ovulation in 4 of 5 women, despite restarting the pills (GEST 75 μg/EE_2 20 μg).

Mid-cycle charcoal treatment on three consecutive days did not alter the bioavailability of GEST or norethisterone (NET), as indicated by measurements of 24-h area under the curve ($AUC_{0\text{-}24h}$), C_{max} and t_{max}. Neither was ovulation detected after treatment with activated charcoal in 11 women using a combination of either 75 μg GEST or 1 mg norethisterone acetate (NET Ac), and 30 μg EE_2. Hence, enterohepatic recirculation of GEST and NET does not seem to be of any clinical significance. This indicates that women using combined oral contraceptives can take activated charcoal for treatment of diarrhea, when administered three hours after pill intake at the earliest, and no later than 12 hours before the next pill.

I INTRODUCTION

The initial prerequisite for development of combined oral contraceptives (OCs) was created far back in the 1930s, when the cybernetic mechanism of regulation between the hypothalamus, pituitary gland and the gonads was recognized (Hannse, 1987). Ethinylestradiol (EE_2) was synthesized by Inhoffen in 1938 and the first synthetic progestogen, norethisterone, by Drejassi in 1951 (Hannse, 1987; Edgren, 1991). The first OC investigated in large-scale clinical trials was, however, a combination that contained 150 μg mestranol and 9.85 mg norethynodrel, marketed in 1960 as Enovid® (Edgren, 1991). Norethynodrel has since vanished from the OC market, since in the early 1960s the conjugated form, norethisterone, showed its superiority (Cook *et al*., 1972; Edgren, 1991). The first OC containing EE_2 (4 mg norethisterone acetate and 50 μg EE_2) was introduced in 1961 as Anovlar® (Hannse, 1987).

In an attempt to minimize the severe consequences of OCs, such as stroke, deep venous thrombosis and lung embolism, and post-pill amenorrhea, hormone doses have gradually been reduced, at first more successfully with regard to the estrogen component. The daily dose of EE_2 has decreased over the years to 15 μg. Along with the development of new, more potent progestogens, the dose of the progestogen component could also be reduced. In this thesis, regimens that contain a daily EE_2 dose of 20-35 μg are referred to as "low-dose" OCs, to differentiate them from older OCs containing 50 μg EE_2, and from OCs with only 15 μg EE_2, the latter being referred to as "ultra low-dose" OCs.

Combined oral contraceptives exert their contraceptive action mainly through inhibition of ovulation via the suppression of gonadotropin levels. Whether this is modulated primarily at the hypothalamus or at the pituitary, is still not completely understood (Hemrika *et al.*, 1993). The suppressive effect of the pill is dose-dependent (Dericks-Tan *et al.*, 1976), and some residual ovarian activity has been demonstrated even with old-type pills containing 50 μg EE_2 (Elstein *et al.*, 1974). However, the results of large clinical studies on OCs containing only 20 μg of EE_2 suggest almost no reduction in contraceptive efficacy (Endrikat *et al*., 1995; Endrikat *et al*., 1997; Endrikat *et al*., 2001; Rosenberg *et al*., 1999). In studies of low-dose OCs, in which ovarian function was determined, a variable degree of ovarian activity was measured, accompanied with almost no ovulation, when compliance was good (Coney and

DelConte, 1999; Crosignani *et al.*, 1996; Fitzgerald *et al.*, 1994; Killick *et al.*, 1998; Spona *et al.*, 1996a; Spona *et al.*, 1996b; van Heusden and Fauser, 1999). In several studies demonstrating noncompliance with OCs containing 30 μg of EE_2 or more, the results have been inconclusive. In addition, treatment groups have been small and few comparisons have been made between different regimens. Furthermore, neither prior to nor after the present study, has noncompliance been tested in women using OCs with less than 30 μg of EE_2.

While noncompliance is probably of the most importance, contraceptive efficacy of the pill may be influenced by any factor which interferes with circulating blood levels of exogenous estrogen or progestogen or with their action at the cellular level (Fraser and Jansen, 1983; Shenfield, 1986). Gastroenteritis has long been considered as a cause of malabsorption, with a consequent potential risk of pill failure (Adlercreutz *et al.*, 1979; Hansen and Lundvall, 1997; John and Jones, 1975; Sparrow 1998). A number of environmental factors may affect the serum concentrations of OC steroids, but evidence of the real clinical importance of any of the factors is scanty (Stadel *et al.*, 1980).

Concurrent medication may affect contraceptive efficacy by changing the pharmacokinetic pathways, affecting absorption and distribution of steroids in body tissues, their metabolism and transport in the circulation, and enterohepatic recirculation and excretion (Brodie and Feely, 1988). With regard to drug interactions and their role in pill failure, those due to induction of hepatic microsomal enzymes are well established (Back *et al.*, 1980a; Crawford *et al.*, 1990; Joshi *et al.*, 1980a). In contrast, the association between broad-spectrum antibiotics and pill failure remains controversial. Such antibiotics may interfere with the enterohepatic circulation of exogenous steroids, thus lowering circulating blood steroid levels (Adlercreutz *et al.*, 1979). However, enterohepatic circulation is thought to be relevant only for EE_2, and few clinical studies have been carried out to investigate enterohepatic circulation and its clinical importance with regard to progestogens.

The present study was aimed at elucidating some of the conflicting areas as regards the risk of escape ovulation under treatment with combined oral contraceptives, to allow us to provide fuller information to women using OCs.

II REVIEW OF THE LITERATURE

1 MECHANISM OF ACTION OF OCS

Although ovulation alone does not necessarily mean pill failure, inhibition of ovulation is considered to be a primary mechanism of the action of OCs. The mid-cycle surge of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), as well as the preovulatory estradiol peak, believed to be the positive feedback mechanism for the release of gonadotropin-releasing hormone (GnRH), do not occur (Mishell *et al.*, 1977). Whether this is regulated primarily at the hypothalamus or higher in the central nervous system by interfering with the pulsatile secretion of GnRH, or by reducing the synthesis and/or release of gonadotropins at the pituitary gland, or both, is not completely understood.

In several early studies, in which this has been investigated by using GnRH challenge tests, results have been conflicting (Mishell *et al.*, 1977; Perez-Lopez *et al.*, 1975; Rubinstein *et al.*, 1978; Römmler *et al.*, 1985; Vanderberg *et al.*, 1974). Neither has the situation been elucidated in a more recent study, where pulsatile secretion of LH was investigated during the use of OCs (Hemrika *et al.*, 1993). However, it was shown that the LH pulse pattern is strongly modified by feedback effects of the progestogen component of OCs.

Although both the estrogen and progestogen components participate in pituitary-ovarian suppression, the overall effect probably being synergistic (Mishell *et al.*, 1977, Römmler *et al.*, 1985), it is generally accepted that ovulation prevention, through inhibition of the LH surge, is mainly generated by way of the progestogen component. The effect seems to be determined by the type and dose of progestogen (Dericks-Tan *et al.*, 1976), as well as the duration of administration (Lähteenmäki, 1978; Römmler *et al.*, 1985).

Similarly to endogenous estrogen, EE_2 may participate in inhibition of pituitary gonadotropin function only within a certain dose range. Even alone, at daily doses of 80 to 100 µg, EE_2 is capable of impairing pituitary reactivity to GnRH release from the hypothalamus (Dericks-Tan *et al.*, 1976; Römmler *et al.*, 1985). Whether or not EE_2 can act at much lower doses is unclear, with conflicting results (Römmler *et al.*, 1985; Vanderberg *et al.*, 1974). Oral contraceptives may also directly affect the ovaries, by depressing ovarian responsiveness to

gonadotropins (Mishell *et al.*, 1971) or by inhibition of ovarian steroid biosynthesis (Aden *et al.*, 1998; Kuhl, 1996).

In addition to ovulation inhibition, OCs prevent normal proliferation of the endometrium (Chowdhury *et al.*, 1980) and, more importantly, they change the cervical mucus, making it less penetrable to semen (Chowdhury *et al.*, 1980; Hamilton and Hoogland, 1989; Killick *et al.*, 1990; Morris *et al.*, 1979; Spona *et al.*, 1993). The former is affected by both the estrogenic and progestogenic components of the pill, whereas the latter results from the progestogenic effect on the mucus.

2 CHARACTERISTICS OF OC STEROIDS

Ethinylestradiol is still the most widely used estrogen component of modern OC preparations in the world. Of the progestogens, norethisterone, levonorgestrel, gestodene, desogestrel, norgestimate and dienogest are 19-nortestosterone derivatives. Apart from dienogest, which was introduced latest, in 1991, these are still the most widely used progestogen components in OCs. Among them, levonorgestrel, gestodene, desogestrel and norgestimate belong to the subgroup of 13-ethyl-gonanes, as they contain a 13β-ethyl group. These derivatives are also called levonorgestrel-family progestogens (Edgren and Stanczyk, 1999) as well as 'gonanes', gonane being the basic structure of nearly all steroid hormones, including natural estrogens, progesterone, androgens and the corticosteroids (Kuhl, 1996).

Norethisterone and dienogest belong to the norethisterone family of progestogens, ‘estranes’, which can also be characterized as 13-methyl-gonanes. Norethisterone acetate, ethynodiol diacetate and lynestrenol, which are still used in some OC formulations, are rapidly and completely converted to norethisterone (Kishimoto *et al.*, 1972; Shrimanker *et al.*, 1980).

Whereas 19-nortestosterone derivatives in general retain varying degrees of androgenic activity, of the gonanes, gestodene, 3-keto-desogestrel and norgestimate have minimal androgenicity (Coenen *et al.*, 1995; Janaud *et al.*, 1992; Philips *et al.*, 1990). Whilst gonanes have an ethinyl group at position 17α, dienogest has a 17α-cyanomethyl group, resulting in antiandrogenic properties (Kuhl, 1996). The 19-norprogesterone derivative nomegestrol (Kuhl, 1996) and the 17α-hydroxyprogesterone derivatives cyproterone and its acetate also

have antiandrogenic effects (Frey, 1975), the latter being used favorably in the treatment of hyperandrogenic symptoms (Erkkola *et al.*, 1990). Drospirenone, a derivative of 17α-spirolactone, has a unique pharmacological profile, notably antimineralocorticoid properties, which make it resemble endogenous progesterone (Oelkers, 2000). The 'evolution' of the current progestogens used in OCs is shown in Figure 1.

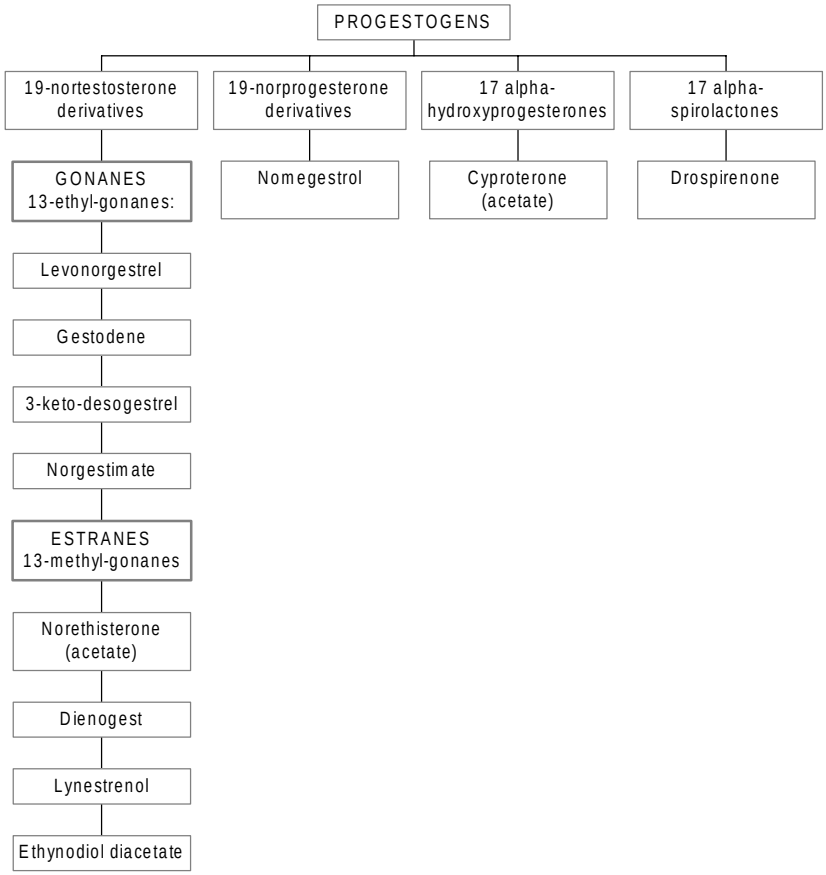

Figure 1 Current oral contraceptive progestogens

3 FACTORS AFFECTING THE RELIABILITY OF OCS

3.1 Composition of the pill

With reduction of steroid doses in OCs, the potency of the progestogen component becomes increasingly important for contraceptive reliability. In only a few clinical studies has the relative importance of the amount of EE_2 versus progestogen, with respect to their potency in inhibiting ovulation, been elaborated, and the results have been conflicting. In a relatively recent study of three different OC regimens (20 μg EE_2 /75 μg gestodene, 20 μg EE_2 /150 μg desogestrel, and 30 μg EE_2/150 μg desogestrel) it was concluded that EE_2 content rather than the progestogenic component determined the extent of residual ovarian activity at the beginning of the pill-free period (van Heusden and Frauser, 1999). In another study, greater follicular activity was observed in women using a combination of 150 μg of desogestrel and 20 μg of EE_2, compared with women on a preparation of 150 μg desogestrel and 30 μg of EE_2 (Mall-Haefeli, 1991). This also suggests a role of EE_2 in the pituitary-ovarian suppression. However, in a large randomized double-blind comparison of two OCs containing 75 μg of gestodene combined with either 20 μg (n=428) or 30 μg (n=211) of EE_2, two of three pregnancies occurred in the latter group, indicating comparable reliability with either preparation (Endrikat *et al*., 1997). Similarly, in almost 5000 cycles with two preparations, one containing 20 μg EE_2 and 150 μg desogestrel and the other containing 30 μg EE_2 and 150 μg desogestrel, 0 and 2 pregnancies occurred, respectively (Åkerlund, 1997).

On the other hand, when the EE_2 dose was fixed at 50 μg per day, divergent responses of LH and FSH during GnRH stimulation have been observed, depending on the type and dose of the progestogenic component (Römmler *et al*., 1985). Only a few large comparative studies evaluating the efficacy of different OCs have been carried out with preparations having 20 μg EE_2. No significant difference was detected between preparations containing 20 μg EE_2/75 μg gestodene, and 20 μg EE_2/150 μg desogestrel (Endrikat *et al*., 1995). Neither was there any difference in pregnancy rate between two OC regimens, both involving 20 μg EE_2 and 100 μg levonorgestrel for 21 days, followed by either 7 hormone-free days or only 2 hormone-free days and then 5 days of 10 μg of EE_2 (Rosenberg *et al*., 1999). When a regimen of 20 μg EE_2/100 μg levonorgestrel was compared with an OC regimen with 20 μg

EE_2/500 μg norethisterone, the Pearl indices were 0.9 and 1.9, respectively (Endrikat *et al*., 2001).

In more closely monitored studies assessing ovarian activity during regular use of OCs containing either 20 μg EE_2/150 μg desogestrel or 20 μg EE_2/75 μg gestodene, no ovulation was detected (Crosignani *et al*., 1996; Fitzgerald *et al*., 1994; van Heusden and Frauser, 1999). In addition, similar levels of residual ovarian activity were demonstrated with both preparations. However, ovarian activity appeared to be more suppressed with an OC that contained 20 μg EE_2 and 150 μg of desogestrel, for 21 days, if followed by only 2 pill-free days and 5 days of 10 μg EE_2, in comparison with one followed by 7 pill-free days (Killick *et al*., 1998). More pronounced ovarian suppression was also observed with a 20 μg EE_2/75 μg gestodene preparation, if taken for 23 days followed by 5 pill-free days, compared with the traditional 21/7 regimen (Spona *et al*., 1996a). With a combination of 20 μg EE_2 and 100 μg levonorgestrel, one ovulation was detected in a study on 26 women (Coney and DelConte, 1999). In another study, with the same combination, 24 women showed no escape ovulation, although a follicle-like structure larger than 13 mm in diameter was detected in half of the women (Spona *et al*., 1996b). With an ultra-low dose regimen of only 15 μg of EE_2 and 60 μg of gestodene one ovulation was detected among 75 regular cycles of 21-day pill-taking (Sullivan *et al*., 1999).

Triphasic preparations contain lower amounts of steroids at the beginning of the pill pack, right after the pill-free interval, which itself confers follicular maturation. It has therefore been suggested that triphasic OCs would result in less complete pituitary-ovarian suppression, and hence an increased risk of functional ovarian cysts, compared with the use of monophasic preparations. However, this view is largely derived from case-reports and uncontrolled studies (Caillouette and Koehler, 1987; Kovacs *et al.*, 1989; Römmler *et al.*, 1985; Smith *et al.*, 1986; Van der Vange *et al.*, 1985), whereas results from more strictly controlled studies do not support it (Crosignani *et al.*, 1996; Gaspard *et al.*, 1984; Grimes *et al.*, 1994; Holt *et al.*, 1992*;* Killick *et al.*, 1990; Lete and Morales 1997; Spona *et al.*, 1993*;* Young *et al.*, 1992).

3.2 Absorption and bioavailability of OC steroids

All factors which decrease either direct absorption of steroids, or their re-entry from the intestine to the circulation (enterohepatic recirculation), may adversely influence pill efficacy. Vomiting soon after pill intake may obviously prevent initial absorption of contraceptive steroids.

After oral administration, steroids in OCs are absorbed as free or conjugated (mainly with sulfate) within the small intestinal wall. It is well established that orally given EE_2 is substantially metabolized by the intestine, reducing its bioavailability by more than 50% (Back *et al.*, 1979; Back *et al.*, 1982a). Norethisterone also undergoes considerable first-pass metabolism *in vivo* during passage through the intestinal wall, resulting in overall bioavailability of 64% (Back *et al.*, 1978b).

Desogestrel, which is a prodrug, is rapidly transformed to its active metabolite 3-keto-desogestrel (Madden *et al.*, 1989; Viinikka, 1979). Norgestimate may also be a prodrug, at least partly: it is metabolized initially to levonorgestrel-3-oxime, but it probably exerts its pharmacodynamic effect mainly through its metabolite levonorgestrel (Kuhl, 1996; Kuhnz *et al.*, 1993b; Madden and Back, 1991). Norgestimate and desogestrel are metabolized by the gut wall *in vitro* (Back *et al.*, 1990; Madden and Back, 1989), but as both progestogens are readily and almost completely metabolized to their active metabolites, there is no accurate data on *in vivo* bioavailability (Hasenack *et al.*, 1986; Kuhnz and Gieschen, 1998). However, the estimated bioavailability of norgestimate is around 60% (McGuire *et al.*, 1990) and mean (±SD) bioavailability figures of 76±22% (Back *et al.*, 1987) and 62±7% (Orme *et al.*, 1991) have been reported for desogestrel.

With gestodene (Kuhl *et al.*, 1988b; Täuber *et al.*, 1990) and levonorgestrel (Back *et al.*, 1981b; Hümpel *et al.*, 1978), which are active drugs themselves, nearly 100% bioavailability has been described. Whether the compound is a prodrug or active itself is probably not of great importance with regard to its pharmacological profile (Hammerstein, 1990).

3.3 Enterohepatic recirculation of OC steroids

Absorbed free steroid may be conjugated by the liver with glucuronic acid or hydroxylated by oxidative liver enzymes. Variable amounts of conjugated steroids, mainly sulfates, are excreted in the bile, released into the colon, hydrolyzed by intestinal microflora, and then reabsorbed as free steroid back to the portal venous system and to the liver (Orme *et al.,* 1983). This process is called enterohepatic recirculation and is illustrated in Figure 2.

Both intestinal microflora, and mucous cell metabolism are thought to be responsible for the metabolism and reabsorption of steroids from the intestine (Adlercreutz *et al.*, 1979). In severe diarrhea, both accelerated motility of the bowel and the degree of inflammation in the gut wall influence the overall effect of the pill. Since the gut microflora is largely responsible for hydrolysis of steroid conjugates, antibiotic treatment, most notably ampicillin and tetracycline, may lead to an increased fecal loss of steroid conjugates and consequently a decrease in the concentrations of circulating free steroids.

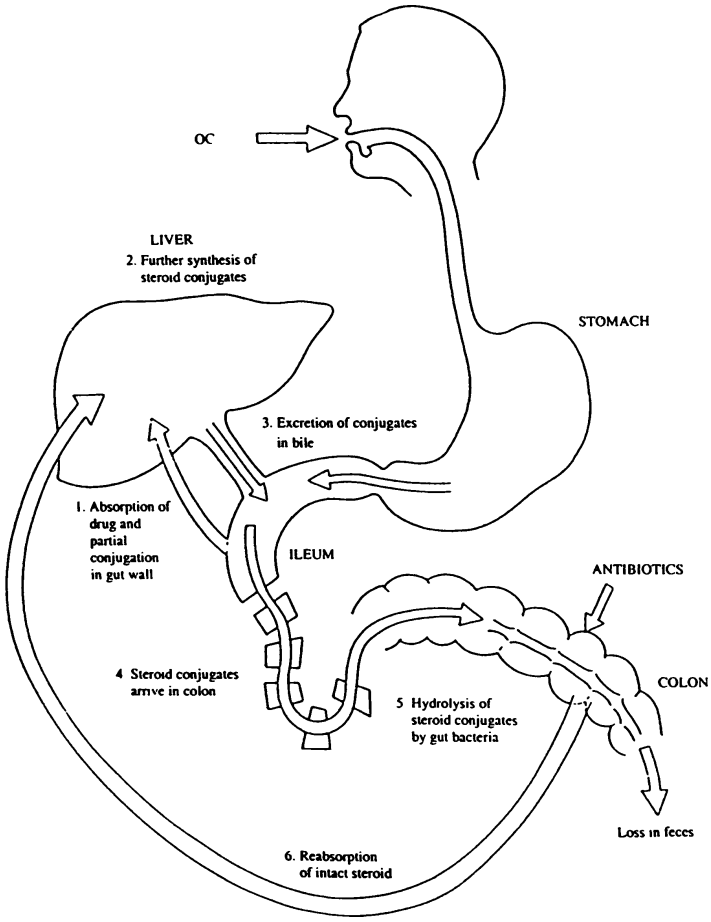

Figure 2 **Schematic representation of the enterohepatic circulation of orally administered OC steroids. (*Adopted from:* Orme and Back, 1986, with permission)**

Enterohepatic circulation has been clearly demonstrated for EE_2 (Back *et al.*, 1979; Back *et al.*, 1980b; Back *et al.*, 1981b). It has also been speculated that enterohepatic circulation may be relevant only for EE_2, because it is directly conjugated at position 3, unlike progestogens (Orme and Back, 1990). The ethinyl group at the 17-position, present both in EE_2 and in gonane progestogens, has been thought to hinder conjugation and, thus, the enterohepatic circulation of progestogens (Back *et al.*, 1981a). However, in man, glucuronidation at the 17-position has been shown to occur extensively in both EE_2 (Sahlberg *et al.*, 1981) and norethisterone (Braselton *et al.*, 1977). In the rat it has been demonstrated that antibiotic treatment reduces enterohepatic cycling of norethisterone, even though there was no evidence of direct conjugation (Back *et al.*, 1978a; Back *et al.*, 1980c).

Clinical evidence as regards the enterohepatic circulation of progestogens is negligible and conflicting. Double peaks of serum EE_2 and norethisterone have been documented between 4–7 hours after oral administration of the steroids, suggesting their enterohepatic recirculation (Zacur *et al.*, 1992). The bioavailability of levonorgestrel has been studied in women with ulcerative colitis, but the results have been inconclusive (Nilsson *et al.*, 1985). Women with mild ulcerative colitis, who were treated by proctocolectomy and continent ileostomy, showed lower serum levonorgestrel concentrations than did healthy women serving as controls ($p<0.05$). In another study, the mean bioavailablity of levonorgestrel was not statistically significantly decreased in women who had undergone ileostomy following lower bowel surgery (Grimmer *et al.*, 1986).

3.4 Plasma concentrations and distribution of OC steroids

As regards all contraceptive steroids, there are many-fold intersubject and interpopulation differences in serum levels (Back *et al.*, 1979; Kuhl *et al.*, 1988a; Kuhl *et al.*, 1988b; Shi *et al.*, 1987). A number of environmental factors may affect the serum concentrations of OC steroids, but less than 30% of the variation has been explained by factors such as lifetime use of OCs, time since ingestion of the last pill, day of the menstrual cycle, race, age, weight, height, blood pressure, alcohol and cigarette consumption and diurnal variation (Stadel *et al.*, 1980). In an individual woman, however, serum levels remain relatively constant from cycle to cycle (Kuhl *et al.*, 1988a; Kuhl *et al.*, 1988b).

In general, the maximum serum concentration (C_{max}) values of contraceptive steroids are reached within 1–2 hours of oral intake (Orme *et al.*, 1983). The time interval to peak concentration (t_{max}) of active metabolites after oral ingestion of a prodrug occurs about one hour later than that of the active drug itself (Hammerstein, 1990; Pasqualini *et al.*, 1977; Sarkar *et al.*, 1983). This, however, does not affect the efficacy of contraceptive steroids as long as absorption of the drug is complete.

All contraceptive steroids are bound to carrier proteins in the plasma and only the unbound fraction, less than 5% of the total, is considered biologically active (Hammond *et al.*, 1984; Orme *et al.,* 1983). Around 95–98% of plasma EE_2 is bound, virtually all to albumin, whereas progestogens are bound to both albumin and sex hormone-binding globulin (SHBG).

Progestogens differ in their potency to inhibit ovulation, partly due to their different binding affinities to plasma proteins (Kuhl, 1996). Gestodene and levonorgestrel are 98% bound, gestodene more notably to SHBG (70–80% after multiple doses), and levonorgestrel slightly more to albumin (Fotherby, 1990; Hammond *et al.*, 1984; Kuhnz *et al.*, 1992; Kuhnz *et al.*, 1993a). More than 60% of 3-ketodesogestrel and norethisterone are bound to albumin and 30–35% to SHBG (Fotherby, 1990; Hammond *et al.*, 1994; Orme *et al.,* 1983), thus showing lower affinity to carrier proteins, because binding to albumin appears to be much weaker than to SHBG (Orme *et al.,* 1983; Fotherby, 1990).

Of all progestogens, gestodene has been considered to be biologically the most potent, probably because it binds largely to SHBG (Back *et al.*, 1993; Hammond *et al.*, 1994; Kuhl, 1996). A lower dose of gestodene results in similar or higher serum concentrations when compared with other progestogens (Back *et al.*, 1993; Hammond *et al.*, 1994). However, serum concentration alone does not always correlate with biological potency, if expressed as potency to prevent ovulation (Fotherby, 1990; Gillmer, 1987). For example, dienogest, with a low affinity to progesterone receptor and relatively high serum concentrations, has a strong effect on the endometrium and cervical mucus, but relatively low ovulation-inhibiting potency (Kuhl, 1996). Gestodene has been demonstrated to be the most potent compound in terms of the lowest dose required for ovulation inhibition (40 μg vs. 60 μg levonorgestrel and desogestrel, 400 μg norethisterone) and it is used at the lowest doses in OCs (60–75 μg, vs. 100–150 μg levonorgestrel and desogestrel, 1000 μg norethisterone) (Fotherby, 1990; Kuhl, 1996; Spona *et al.*, 1993).

Ethinylestradiol itself induces hepatic synthesis of SHBG, thus leading to increased serum SHBG-bound progestogen and, consequently, a decreased free fraction. Levonorgestrel, as an antiestrogenic progestogen, can oppose this effect (Victor and Johansson, 1977), whereas others such as gestodene and desogestrel lack this property (Hammond *et al.*, 1984).

The higher the binding affinity to SHBG the stronger the distribution towards the SHBG-bound fraction. Hence, gestodene is more affected by EE_2-induced SHBG synthesis than progestogens with less affinity to SHBG. Although only a non-protein-bound progestogen is considered to be biologically active, there is no evidence that the relative decrease of free

progestogen results in impairment of contraceptive efficacy (Fotherby, 1990; Hammond*et al.*, 1994). Regarding gestodene, this may be explained by the relatively very high serum concentrations of both the free and protein-bound components achieved after a considerably smaller dose compared with other progestogens. Furthermore, strong binding to SHBG may restrict the distribution of gestodene to other body fluids and tissues (Orme *et al.*, 1991).

3.5 Accelerated metabolism and excretion

Gonanes are considered to be much more potent than estranes, as the 13-ethyl group of gonanes protects them against rapid liver metabolism through inhibition of cytochromal P450 (CYP) enzymes in the liver; more specifically 5α-reductase and CYPIIIA4 isoenzymes (Kuhl, 1996). This inhibition of CYP enzymes may contribute to impairment of follicular development and prevention of ovulation.

While most of the OC steroid conjugates excreted in bile are sulfates, in urine they are excreted chiefly as glucuronides and to a lesser extent as sulfates (Orme *et al.,* 1983). As explained in section 3.3, impairment of enterohepatic recirculation of OC steroids may lead to excessive fecal elimination of steroid conjugates, and thus to reduction of contraceptive efficacy.

3.6 Noncompliance in pill taking

In a interview study among 769 ‘reliable pill takers’, noncompliance in pill taking was excluded on the basis of the results of a questionnaire which was completed at the time a woman was attending for termination of pregnancy (Sparrow, 1998). One third of the women who experienced accidental pregnancy during use of an OC or progestogen-only pills did not report any known predisposing factor such as gastroenteritis or drug interaction. These cases were thus treated as method failures. In two thirds of the women who reported a factor interfering with their OC, pregnancy was most often associated with vomiting and/or diarrhea (39%) and with concurrent antibiotic treatment (21%). Over a quarter (28%) of the women had also experienced previous pill failure.

In another study, 800 OC failures were identified from hospital records among 8192 women who had undergone pregnancy termination. Eventually, after validation of the data, one third of these 800 women appeared not to have been using an OC at the time of conception (Skjeldestad, 2000).

Studies in different populations demonstrate great variation (3–60%) in the proportions of oral contraceptive users who self-report missed pills (Potter, 1996). However, as in the above-mentioned studies, these studies are based on interviews with women and/or the use of daily diaries in which women have been asked to record their tablet taking. Definitions of 'late' or 'missed' pills are also different from study to study (Potter, 1996). In general, up to 20% of women report that they have omitted two or more pills per cycle and almost half of the women on OCs miss one or more pills in each cycle (Rosenberg *et al.*, 1995; Rosenberg *et al.*, 1998).

Only recently, by way of use of electronic pill dispensers, augmented with a precoded microchip, has it been shown that the real incidence of missed pills is much higher than reported by women, and the discrepancy also increases over time (Potter *et al.*, 1996). During the first two treatment cycles the electronic data showed that over 30% of women forgot ≥3 pills, whereas only 10% of women reported that they had forgotten that number. During the third cycle it was recorded electronically that more than 50% of users missed ≥3 pills, compared with self-reports of only 14% (Potter *et al.*, 1996).

As shown in Table 1, in studies in which noncompliance has been simulated by timed omission of 1 to 4 pills, no ovulation has been demonstrated almost exclusively (Hamilton and Hoogland, 1989; Hedon *et al.*, 1992; Letterie and Chow, 1992; Morris *et al.*, 1979; Nuttal *et al.*, 1982; Wang *et al.*, 1982). In one study, however, almost one third of the women ovulated after deliberate omission of two consecutive pills between cycle days 5–17 (Chowdhury *et al.*, 1980). Ovulation was determined by elevation of serum progesterone of >4 ng/ml. It was suggested that the high failure rate could be explained either by the OC regimen used in the study or by noncompliance. In contrast, in another study, no luteinization was seen during 7 pill-free days which were preceded by only 7 days of pill-taking (Smith *et al.*, 1986).

Table 1 The risk of ovulation after pill omission (excluding extended pill-free periods)

Study	Number of missed pills and time of cycle	No. of ovulations/ No. of subjects	OC cycle	Regimen(s)
Chowdhury *et al.*, 1980	2 consecutive pills anytime between pill days 5-17	10/35 5/19	1st 4th	N=54:35 µg EE_2 + 1.0 mg NET Ac
Hamilton and Hoogland, 1989	1 (pill no. 2)	0/9	2nd	N=9: 35 µg EE_2 + 0.5 mg NET (7 days), 35 µg EE_2 + 0.75 mg NET (7 days), 35 µg EE_2 + 1.0 mg NET (7 days)
Hedon *et al.*, 1992	1-4 pills, started on pill day: 6 12 18	 0/7 0/7 0/10*)	2nd	N=24:35 µg EE_2 + 250 µg NGM
Letterie and Chow, 1992	4 (pills no. 3-6) 4 (pills no. 6-9)	0/5 0/5	Not stated	N=10:35 µg EE_2 + 0.5 mg NET (7 days), 35 µg EE_2 + 0.75 mg NET (7 days), 35 µg EE_2 + 1.0 mg NET (7 days)
Morris *et al.*, 1979	1 (pill no.4) 1 (pill no. 19)	0/7 0/3	3rd or later	N=10:30 µg EE_2 + 150 µg LNG
Nuttal *et al.*, 1982	1 (pill no.10) 2 (pills no. 9-10)	0/6 0/6	2nd 3rd	N=6: 20 µg EE_2 + 250 µg LNG
Wang *et al.*, 1982	2 (pills no. 3-4) 2 (pills no. 6-7) 2 (pills no. 9-10)	0/8 0/8 0/8	4th or later	N=24:30 µg EE_2 + 150 µg LNG

*) 3 subjects with 4 missed pills starting on pill day 18 are included in Table 3, as the situation is identical to an extended pill-free period.
LNG = levonorgestrel, NET = norethisterone, NET Ac = norethisterone acetate, NGM = norgestimate

3.7 The pill-free interval and residual ovarian activity

In general, a follicle exceeding 10 mm in diameter is considered to be a prerequisite for further development of the follicle, whereas a smaller follicle does not seem to have any activity. A diameter of 13 mm has been thought to be critical in terms of ovulation (Hoogland and Skouby, 1993). Grading of ovarian activity, as determined by the size of a follicle-like structure and serum estradiol and progesterone concentrations, is shown in Table 2 (Hoogland and Skouby, 1993). In the present study all follicle-like structures are consistently called follicles, even though their activity is not possible to determine in ultrasonographic examination and, therefore, a follicle-like structure would be a more accurate term.

Table 2 Grading of follicular activity

Grade of ovarian activity	Follicle-like structure (mm)	S-Estradiol (pmol/L)	S-Progesterone (nmol/L)
0 No activity	≤ 10		
1 Potential activity	> 10		
2 Non-active FLS	> 13	≤ 100	
3 Active FLS	> 13	> 100	≤ 5
4 LUF	> 13, persisting	> 100	> 5
5 Ovulation	> 13, ruptured	> 100	> 5

FLS = Follicle-like structure
LUF = Luteinized unruptured follicle

Ovarian activity, especially during the pill-free period, has been clearly demonstrated even with pills containing 30 µg of EE_2 or more, by way of raised serum gonadotropin and estradiol levels (Cohen and Katz, 1979; Killick *et al.*, 1987; Lähteenmäki and Luukkainen, 1978; Smith *et al.*, 1986) and by detecting follicular development by ultrasonography (Hamilton and Hoogland, 1989; Killick *et al.*, 1987; Tayob *et al.*, 1990; Van der Vange *et al.*, 1985). Whilst follicular activity grading 2 or higher (see Table 2) counts in 23 - 37% of cycles of women using OCs containing 30 µg of EE_2 or more (Hamilton and Hoogland, 1989; Tayob *et al.*, 1990), the grade 2 - 5 has been detected in 9 - 60% of the cycles with low-dose preparations of only 20 µg EE_2 (Crosignani *et al*., 1996; Fitzgerald *et al*., 1994; Spona *et al*., 1996a; van Heusden and Frauser, 1999).

These findings have led to the idea that missing the first pills of the pack, thereby extending the pill-free period, would be critical as regards escape ovulation (Fraser and Jansen 1983; Killick *et al.*, 1990; Landgren and Csemiczky, 1991; Landgren and Diczfalusy, 1984; Wang *et al.*, 1982). However, in studies in which noncompliance has been simulated by extending the pill-free period to 8–11 days, the results have been inconclusive. Whilst in one study only the first pill was omitted during the second pill cycle and one of nine women ovulated (Hamilton and Hoogland, 1989), no ovulation was detected after an 11-day pill-free period (Hedon *et al.*, 1992; Killick *et al.*, 1990; Letterie and Chow, 1992). Studies in which pituitary-ovarian inhibition has been challenged by extending the pill-free period are presented in Table 3. In these studies both mono- and triphasic preparations have been used, with a variety of progestogens and with a minimum daily EE_2 dose of 30 µg.

In one study, the duration of the pill-free period was extended until a predetermined size of dominant follicle was reached, as detected by ultrasonography (Killick, 1989). At the stage when the leading follicle had reached a diameter of 12 mm (in 7–16 days in individual women), the women were asked to resume pill-taking. Eight of ten women continued to develop preovulatory follicles of 18 mm in diameter, and all eight women ovulated within 48 hours after administration of human chorionic gonadotropin (HCG). It was concluded that ovulation was induced by HCG and that pill omissions may lead to ovulation if they take place at the stage when follicular development is optimal (Killick, 1989). In another study, a regimen of 50 µg EE_2 and 1 mg norethisterone for 5 days, followed by 0.7 mg of norethisterone alone for subsequent 9 days, and then by 14 days on placebo was started either 6 days (group 1) or 8 days (group 2) after the onset of menses (Letterie, 1998). It was claimed that the studied regimen gives effective prevention against pregnancy. During the second cycle, i.e. after resuming the pills, one of five women ovulated in group 1 and all 5 women ovulated in group 2. No data has been published on the effect of additional pill omissions in circumstances in which the extended pill-free period has resulted in a preovulatory follicle.

Table 3 Studies evaluating the risk of escape ovulation resulting from an extended pill-free period

Study	Number of missed first/ last pills of the packet	No. of ovulations/ No. of subjects	OC cycle	Regimen(s)
Dericks-Tan *et al.*, 1980	2	1/5	*)1st	N=9: 37.5 µg EE_2 + 0.75 mg LYN
Hamilton and Hoogland, 1989	1	1/9	2nd	N=9: 35 µg EE_2 + 0.5 mg NET (7 days), 35 µg EE_2 + 0.75 mg NET (7 days), 35 µg EE_2 + 1.0 mg NET (7 days)
Hedon *et al*., 1992	1 2 3 4	0/4 0/4 0/4 0/6	2nd	N=18:35 µg EE_2 + 250 µg NGM
Killick *et al.*, 1990	2 4	0/14 0/14	2nd and 3rd, intra-individual cross-over	N=10: 35 µg EE_2 + 150 µg LNG N=9: 35 µg EE_2 + 150 µg LNG (6 days), 40 µg EE_2 + 75 µg LNG (5 days), 30 µg EE_2 + 125 µg LNG (10 days) N=9: 30 µg EE_2 + 75 µg GEST
Landgren and Diczfalusy 1984	2	0/10	4th or later, 3 consecutive cycles	N=10: 30 µg EE_2 + 150 µg LNG
Landgren and Csemiczky, 1991	3	1/10 1/10	4th or later	N=10: 30 µg EE_2 + 150 µg DSG N=10: 35 µg EE_2 + 150 µg LNG (6 days), 40 µg EE_2 + 75 µg LNG (5 days), 30 µg EE_2 + 125 µg LNG (10 days)
Letterie and Chow, 1992	4	0/5	Not known	N=5: 35 µg EE_2 + 0.5 mg NET (7 days), 35 µg EE_2 + 0.75 mg NET (7 days), 35 µg EE_2 + 1.0 mg NET (7 days)
Wang *et al.*, 1982	2	0/8	4th or later	N=8: 30 µg EE_2 + 150 µg LNG

*)Pills started on day 10 of the initial cycle, DSG = desogestrel, GEST = gestodene, LNG = levonorgestrel, LYN = lynestrenol, NET = norethisterone, NGM = norgestimate

3.8 Drug interactions

Concomitant medication can theoretically reduce the efficacy of OCs by a variety of mechanisms. It may interfere with the initial absorption of OC steroids or with their reabsorption, i.e. enterohepatic recirculation, induction of CYP enzyme activity in the liver, or serum protein binding capacity of OC steroids. Finally, urinary excretion of OC steroids may be increased by concurrent medication (DeSano and Hurley, 1982).

The first report on the possible loss of contraceptive efficacy described increased intermenstrual bleeding in OC users who had concurrently received rifampicin and other antituberculous drugs (Reimers and Jezek, 1971). Since then numerous case reports have been documented and experiments performed to investigate the underlying mechanisms of drug interactions.

Rifampicin (Joshi *et al.*, 1980a), rifabutin (Barditch-Crovo *et al.*, 1999) and certain anticonvulsants, such as barbiturates and their derivatives, phenytoin, carbamazepine and their derivatives (Back *et al.*, 1980a; Crawford *et al.*, 1990) are capable of inducing CYPIIIA4 isoenzymes in the liver, which participate in the 2-hydroxylation of EE_2. The carbamazepine derivative oxcarbazepine, as well as felbamate and topiramate, may also induce hepatic CYP enzymes, although to a lesser extent (Jensen *et al.*, 1992; Larkin *et al.*, 1991; Wilbur and Ensom, 2000). The second-generation antiepileptic agents gabapentin, lamotrigine, tiagabine and vigabatrin, may be given to OC users without significant pharmacokinetic interactions (Bartoli *et al.*, 1997; Wilbur and Ensom, 2000). Pregnancies during use of the antituberculous drug isoniazid are likely to be a result of concurrent use of rifampicin (Joshi *et al.*, 1980a).

The same agents that induce CYP enzymes in the liver are capable of increasing SHBG binding capacity for progestogens, thereby resulting in a decrease in their free plasma concentrations (Back *et al.*, 1980d; Orme and Back 1980; Victor *et al.*, 1977). Whether this leads to a reduction in contraceptive efficacy has not yet been proven.

Broad-spectrum antibiotics, such as penicillin, tetracycline and their derivatives, have been thought to interfere with enterohepatic recirculation of OC steroids. There are several anecdotal reports on OC users who have experienced breakthrough bleeding or pregnancy when given broad-spectrum antibiotics (Back *et al.*, 1988; Bacon and Shenfield, 1980;

Bainton, 1986; DeSano and Hurley, 1982). Although ampicillin treatment has been shown to interfere dramatically with the gut bacteria responsible for hydrolysis of steroid conjugates, controlled studies have almost systematically failed to show any association between broad-spectrum antibiotics and pill failure (Back *et al.*, 1982b; Friedman *et al.*, 1980; Joshi *et al.*, 1980b). Similarly, no signs of reduced contraceptive efficacy have been documented during concurrent use of tetracycline derivatives (Murphy *et al.*, 1991; Neely *et al.*, 1991; Orme and Back 1986), erythromycin (Orme and Back 1986), cotrimoxazole (Grimmer *et al.*, 1983), temafloxacin (Back *et al.*, 1991), fluoroquinolone (Csemiczky *et al.*, 1996), roxithromycin (Meyer *et al.*, 1990), or metronidazole (Joshi *et al.*, 1980b). In a retrospective cohort study involving 311 woman-years of use of OCs and concurrent antibiotics, and 1245 woman-years of OC exposure alone, no difference was detected in pregnancy rates (Helms *et al*., 1997).
A few anecdotal cases of unintended pregnancies have been reported in women using H_1 antagonists (DeSano and Hurley, 1982), laxatives (Köhler *et al.*, 1976), griseofulvin (Van Dijke and Weber, 1984), and azol antifungals (Pillians and Sparrow, 1993). However, with the exception of griseofulvin, these agents have been considered to confer no risk during the use of OCs (CSAC, 1989; Hilbert *et al.*, 2001; Lunell *et al.*, 1991; Sinofsky and Pasquale, 1998).

Since charcoal is not absorbed from the gastrointestinal tract, it prevents the absorption of steroids from both the stomach and intestine. This fact has been utilized in the treatment of patients who have ingested poisonous substances. The effect of activated charcoal on the bioavailability of steroids has been previously studied in connection with the antiprogestin mifepristone (RU 486) (Heikinheimo *et al.,* 1989). Activated charcoal was ingested repeatedly, starting 6 hours after oral administration of 200 mg of mifepristone. A clear reduction in serum mifepristone levels was seen on day 1, in comparison with the controls. This suggests impaired enterohepatic recirculation of mifepristone. Significantly reduced plasma estriol concentrations have been measured in menopausal women 2 hours after the intake of 20 g of activated charcoal, when administered three hours after the oral estriol (Heimer and Englund, 1986). In an *in vitro* study, activated charcoal has been shown to be a very powerful adsorbent of steroids (Fadel *et al.,* 1979). In the presence of different adsorbents, i.e. antacids, peptic ulcer drugs, and activated charcoal, the last named reduced the dissolution of norethisterone acetate most efficiently. Antacid did not affect the bioavailability of contraceptive steroids in women using OCs (Joshi *et al.*, 1986).

Drugs that have been reported to reduce the contraceptive efficacy of OCs, suspected mechanisms of interaction, level of documentation and the clinical significance of the interaction are presented in Table 4.

Table 4 Drugs claimed to reduce the efficacy of OCs

Drug	Suspected mechanism	Documentation	Clinically significant	References
Anticonvulsants				
Barbiturates, Carbamazepine, Phenytoin	CYP enzyme induction	Established	Yes	Back *et al.*, 1980a; Crawford *et al.*, 1990
Oxcarbazepine, Felbamate, Topiramate	CYP enzyme induction	Possible	Yes	Jensen *et al.*, 1992; Larkin *et al.*, 1991; Wilbur and Ensom, 2000
Antituberculous drugs				
Rifampicin, Rifabutin	CYP enzyme induction	Established	Yes	Joshi *et al.*, 1980a; Reimers and Jezek, 1971; Barditch-Crovo *et al.*, 1999
Isoniazid	No known mechanism	Doubtful	No	Joshi *et al.*, 1980a
Antimycotics				
Griseofulvin	CYP enzyme induction	Possible	Yes	Van Dijke and Weber, 1984
Azole derivatives	No known mechanism	Doubtful	No	Lunell *et al.*, 1991; Pillians and Sparrow, 1993
Broad-spectrum antibiotics				
Penicillin and derivatives	Decreased reabsorption due to interruption of EHC	Possible	?	Back *et al.*, 1982b; Back *et al.*, 1988; Bacon and Shenfield, 1980; Bainton, 1986; DeSano and Hurley, 1982; Friedman *et al.*, 1980; Joshi *et al*, 1980b
Tetracycline and derivatives	Decreased reabsorption due to interruption of EHC	Possible	?	Murphy *et al.*, 1991; Neely *et al.*, 1991;Orme and Back, 1986
Other antibiotics				
Cotrimoxazole	Decreased reabsorption due to interruption of EHC?/ No known mechanism	Doubtful	No	Grimmer *et al.*, 1983
Erythromycin				Orme and Back 1986
Temafloxacin				Back *et al.*, 1991
Fluoroquinolone				Csemiczky *et al.*, 1996
Roxithromycin				Meyer *et al.*, 1990
Metronidazole				Joshi *et al.*, 1980b
Adsorbents				
Activated charcoal	Decreased absorption and reabsorption	Possible	?	Fadel *et al.,* 1979; Heikinheimo *et al.,* 1989; Heimer and Englund, 1986
Antacids	Decreased absorption and reabsorption	Doubtful	No	Fadel *et al.,* 1979; Joshi *et al.,* 1986
Laxatives				
only strong and fast-acting cathartics	Decreased absorption and reabsorption	Possible	?	Köhler *et al.*, 1976
Antihistamines				
H_1 antagonists	CYP enzyme induction?	Doubtful	No	CSAC, 1989; DeSano and Hurley, 1982

CYP = cytochromal P450, SHBG = sex hormone-binding globulin, EHC = enterohepatic circulation

3.9 Other factors predisposing to pill failure

A higher frequency of spotting or breakthrough bleeding has been reported in smokers who use OCs, compared with non-smoking women on OCs (Rosenberg *et al.*, 1996). It has been suggested that this may be connected to the increased clearance of EE_2 described in smokers (Breckenridge *et al.*, 1980; Kanarkowski *et al.*, 1988). However, in a study with more than 300 women, smoking did not affect the overall rate of either EE_2 or levonorgestrel metabolism (Crawford *et al.*, 1981), and nicotine is known to induce CYPIA2 isoenzyme, whereas EE_2 is metabolized via CYPIIIA4 isoenzyme (Pelkonen *et al*., 1998).

According to some investigators, smoking seems to be strongly associated with inadvertent pregnancies during pill use (Kakouris and Kovacs, 1994; Sparrow, 1998). Kakouris and Kovacs (1994) compared two cohorts, one of OC users who experienced inadvertent pregnancy and one of non-pregnant OC users, over two different time periods, to evaluate predisposing factors associated with pill failure. In the pregnant group, smoking was much more common. There was no difference in the prevalence of any other known risk factor, such as vomiting, diarrhea or concurrent drug use, but there were five times more teenagers in the pregnant group. Hence, it was concluded that pill failure may have occurred more often among smokers not because of smoking *per se* but because of other factors predisposing women to compliance problems, such as young age.

The same probably applies to alcohol consumption and pill failure. Alcohol drinking may confer an increased risk of forgetting pills, but there is no evidence of pharmacological interactions between ethanol and oral contraceptive steroids which may reduce their contraceptive efficacy (Sarkola, 2001; Winstanley and Orme, 1989). No alteration was detected in serum levonorgestrel concentrations in rats receiving oral levonorgestrel after six-week exposure to alcohol (Gomaa *et al.*, 1984). However, following intravenous administration, the elimination half-life of levonorgestrel was shorter and its metabolic clearance rate higher in the ethanol-treated rats than in the control group.

Food may affect drug bioavailability in many ways, but no clinically significant interactions have been shown with OCs (Winstanley and Orme, 1989). The clearance of norethisterone has been found to be accelerated in Indian women of poor nutritional and socio-economic status, compared with women belonging to a high socio-economic group (Prasad *et al.*,

1979). The termination half-life of norethisterone correlated positively with the body mass index of these women. It is unclear whether the shorter termination half-life was due to low body mass index or altered metabolism resulting from malnutrition.

It has been suggested that severe psychological stress and systemic illness, other than gastroenteritis (John and Jones, 1975; Hansen and Lundvall, 1997; Sparrow, 1998) or chronic bowel disease (Grimmer *et al.*, 1986; Nilsson *et al.*, 1985) may affect the utilization of pill steroids (Sparrow, 1998). However, it is not known how severe stress might affect pill efficacy.

Ovarian suppression has been shown to be greater in women on OCs who are over 35 years of age (Fitzgerald *et al.*, 1999). This is due to an age-dependent increase in serum FSH levels which reflects increased ovarian resistance to follicular development.

When OCs that contained EE_2 and norethisterone were administered at various times of the day, no association was detected between a number of pharmacokinetic parameters and the different times of administration (Kiriwat and Fotherby, 1983). If OCs are taken regularly, no difference is expected in their efficacy during the initial cycle or later pill cycles, even though steady serum concentrations of OC steroids are established only after 1–3 months of use (Kuhl *et al.*, 1988b; Kuhnz *et al.*, 1993a).

Factors other than drug interactions that have been thought to relate to pill efficacy are summarized in Table 5.

Table 5 Factors reported to reduce the efficacy of OCs, other than drug interactions

Factor	Suspected mechanism	Documentation	Clinically significant	References
Noncompliance in pill taking	Low serum steroid levels	Established	Yes	See Tables 1 and 3
Vomiting	Decreased absorption	Established	Yes	Adlercreutz *et al.*, 1979;
Diarrhea	Decreased absorption and reabsorption due to interruption of EHC	Possible	?	Hansen and Lundvall, 1997; John and Jones, 1975; Sparrow, 1998
Chronic bowel disease	Decreased reabsorption due to interruption of EHC	Doubtful	No	Grimmer *et al.*, 1986; Nilsson *et al.*, 1985
Systemic illness/stress	No known mechanism	Doubtful	No	Sparrow, 1998
Smoking	CYP enzyme induction	Doubtful	No	Breckenridge *et al.*, 1980; Crawford *et al.*, 1981; Kakouris and Kovacs, 1994; Kanarkowski *et al.*, 1988; Rosenberg *et al.*, 1996; Sparrow, 1998
Acute or chronic use of alcohol	CYP enzyme induction?	Doubtful	No	Gomaa *et al.*, 1984; Sarkola, 2001; Winstanley and Orme, 1989
Diet	Delayed or decreased absorption	Doubtful	No	Winstanley and Orme, 1989
Poor nutrition/ low BMI	Altered metabolism, shorter elimination half-life?	Doubtful	No	Prasad *et al.*, 1979
Ethnic factors	Different liver metabolism	Possible	?	Stadel *et al.*, 1980
Time since initiation of OCs	Delayed steady-state serum steroid concentrations	Doubtful	No	Kuhl *et al.*, 1988b; Kuhnz *et al.*, 1993a
Time of administration	Diurnal variation of metabolism	Doubtful	No	Kiriwat and Fotherby, 1983

EHC = enterohepatic circulation

III AIMS OF THE STUDY

The present study was aimed at elucidating the risk of escape ovulation during the use of OCs, to allow us to provide fuller information to women using OCs. More specifically they were:

To investigate if there are differences between OC regimens in their potency to inhibit ovulation, when challenged by extending the pill-free period by a fixed number of days.

To assess the ovulatory potential of follicles allowed to grow to a predetermined size, before restarting OC treatment.

To test whether oral administration of activated charcoal could inhibit the enterohepatic recirculation of the oral contraceptive steroids gestodene and norethisterone, thus conferring a risk of escape ovulation during regular OC use.

IV MATERIALS AND METHODS

1 DESIGN OF THE TRIALS, AND TRIAL SITES

The present study was conducted at three centers: the Family Planning Clinic (later: the Sexual Health Clinic) of the Family Federation of Finland (Väestöliitto), Helsinki, Finland (studies I-IV); the Department of Obstetrics and Gynecology, University Hospital St. Radboud, Nijmegen, The Netherlands (study I); and the Department of Obstetrics and Gynecology, University Hospital of Gasthuisberg, Leuven, Belgium (study I).

Of the total of 128 recruited subjects, 125 were enrolled and 115 women completed the study. As the subjects acted as their own controls, of the enrolled subjects only those who completed the study were included in the statistical analyses. In addition to the 10 women who discontinued, one woman had a persisting follicular-like structure from the first monitoring. Her data was therefore removed, leaving a total of 114 eligible subjects for analyses. The disposition of the subjects, with reasons for premature discontinuation, is illustrated in Figure 3.

Adverse events leading to premature discontinuation in two women in study I comprised one case of massive vaginal bleeding lasting one day during the use of monophasic gestodene pill (the subject discontinued after 56 days) and mood changes in a woman on triphasic gestodene pill (withdrew from the study after 23 days). In studies III & IV, mood changes and swelling led to a premature discontinuation of one woman after 30 days' use of monophasic gestodene pill, and another woman using monophasic gestodene pill discontinued her participation in the study after two months because of deep venous thrombosis.

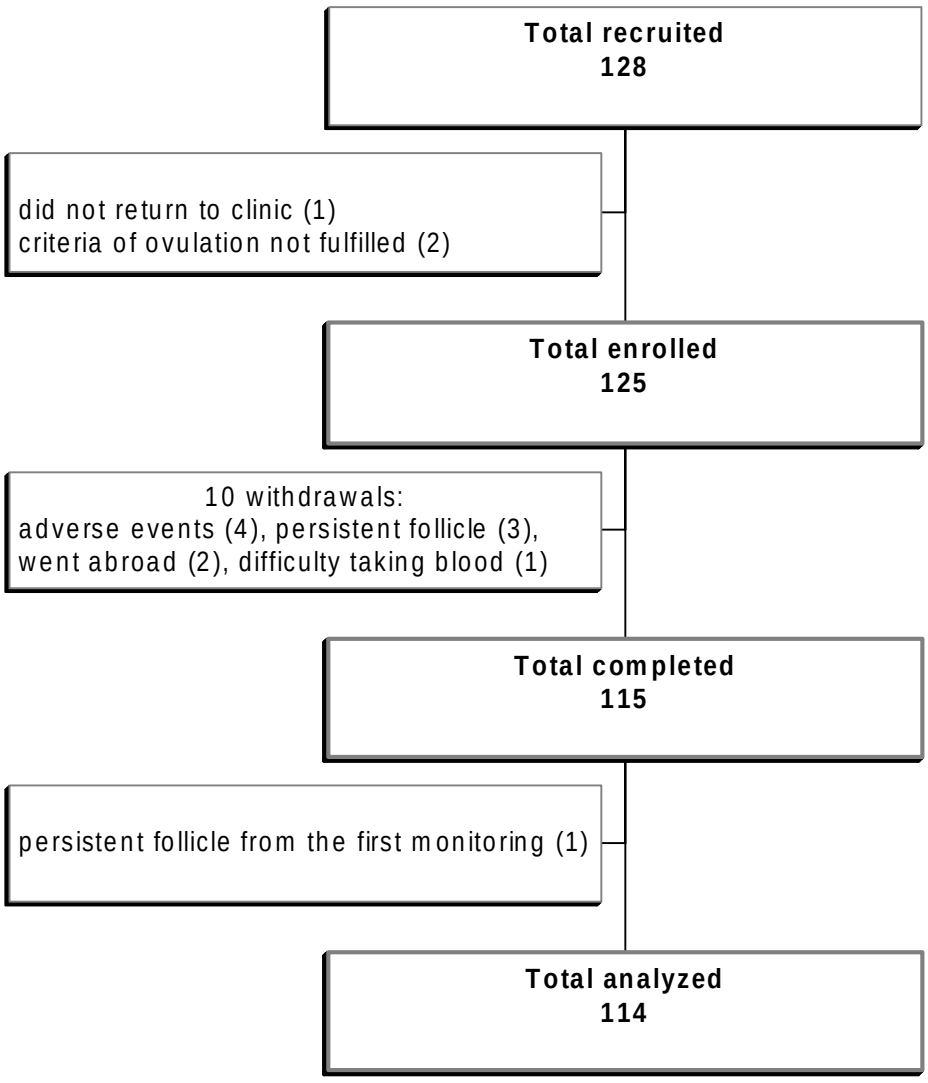

Figure 3 Disposition of the subjects

The volunteers were either regular visitors of the participant clinics or they were recruited by way of a newspaper advertisement. Very similar eligibility criteria were used in all studies. Volunteer subjects were to be 18 to 35 years old, generally healthy, showing no abnormalities in physical or gynecological examinations, or in the cervical smear preceding study enrollment. The women

must have had regular spontaneous menstrual cycles of 21–32 days, and no absolute contraindications for combined oral contraceptive use. In study I only women with confirmed ovulation in one spontaneous cycle before admission were enrolled. Ovulation was documented by elevated serum progesterone levels (>15 nmol/L) during the mid-luteal phase (days 19–23).

Smoking was allowed only in study I, whereas hemoglobin concentration within the normal range was a prerequisite for participation in studies II–IV. Breast-feeding was an exclusion criterion in all studies and pregnancy was excluded prior to study admission either by a pregnancy test or on the basis of menstrual and contraceptive history and the result of a gynecological examination. In all studies the subjects were provided (free of charge) with condoms for their contraception during specified periods of studies when the contraceptive efficacy of the pills was potentially compromised.

Characteristics of the subjects are shown in Table 6 for those who completed the present study. In study I, despite randomization, a statistically significant difference was seen with regard to body mass index (BMI; kg/m^2) between the triphasic gestodene (TG) and monophasic desogestrel (MD) groups. However, this did not affect either follicular growth over 13 mm in diameter, or serum estradiol levels.

Table 6 **Subjects, study designs, methods and main outcomes in the original publications. Data are shown as means ± SD**.

Original publication	**I**	**II**	**III**	**IV**
Study design	phase IV, open randomized, comparative, parallel-group, multi-center	phase IV, open non-randomized, single-group, pharmacodynamic	phase IV, open randomized, intra-individual cross-over, pharmacokinetic comparison	phase IV, open randomized, intra-individual cross-over, pharmacodynamic comparison
Study duration	3 cycles	2 cycles	4+4 cycles	4+4 cycles
Purpose of the study	To assess ovulation risk during and after 7-day and 10-day pill-free periods	To assess ovulation risk of a predetermined sized follicle	To determine bioavailability of GEST and NET after interruption of EHC	To assess ovulation risk after interruption of EHC
No. of subjects by treatment	n=34 MG n=34 TG n=30 MD	n=5 Harmonet®	n=11 Minulet® + Econ/30®	n=11 Minulet® + Econ/30®
Age (years)	MG 26.8 ± 4.2 TG 26.7 ± 4.7 MD 26.3 ± 4.4	23.6 ± 5.0	26.5 ± 5.1	26.5 ± 5.1
BMI (kg/m^2)	MG 21.7 ± 2.2 TG 21.5 ± 3.0‡ MD 23.1 ± 3.9‡	22.1 ± 1.7	22.7 ± 3.0	22.7 ± 3.0
Main methods	Hormone assays, US scanning of ovaries	Hormone assays, US scanning of ovaries, GnRH stimulation test	Hormone assays, US scanning of ovaries, Oral activated charcoal	Hormone assays, US scanning of ovaries, Oral activated charcoal
Main variables	S-FSH, S-Estradiol, S-Progesterone, Follicle diameter	S-FSH, S-LH, S-Estradiol, S-Progesterone, Follicle diameter	S-GEST, S-NET: AUC_{24}, C_{max}, t_{max}	S-FSH, S-LH, S-Estradiol, S-Progesterone, Follicle diameter
Main results	No ovulation, one LUF	4/5 women ovulated, one LUF	No reduction in bioavailability of GEST or NET	No ovulation

MG = monophasic gestodene (Minulet®), TG = triphasic gestodene (Tri-Minulet®), MD = monophasic desogestrel (Mercilon®), EHC = enterohepatic circulation, US = ultrasonography, LUF = luteinized unruptured follicle, GEST = gestodene, NET = norethisterone, ‡ Significant difference (p=0.045)

2 ETHICAL CONSIDERATIONS

The local Ethics Committees approved the protocols and details regarding informed consent before the studies, and the studies were conducted in accordance with the ethical principles of the Helsinki declaration.

Prior to the studies, the volunteers gave signed informed consent, after being informed about the nature and requirements as well as the benefits and potential risks of the studies.

3 STUDY TREATMENTS

In study I the volunteers were randomly allocated to receive one of the following three preparations:

1. Minulet® (Wyeth-Ayerst Laboratories, St. David's, Pa):
   75 μg GEST and 30 μg EE_2 in each pill (referred to also as 'MG');
2. Tri-Minulet® (Wyeth-Ayerst Laboratories, St. David's, Pa):
   50 μg GEST and 30 μg EE_2 (6 days)
   70 μg GEST and 40 μg EE_2 (5 days)
   100 μg GEST and 30 μg EE_2 (10 days) (referred to also as 'TG');
3. Mercilon® (NV Organon, Oss, The Netherlands):
   150 μg desogestrel and 20 μg EE_2 in each pill (referred to also as 'MD').

The above-mentioned OC preparations, as well as Harmonet® (75 μg GEST and 20 μg EE_2 ; Wyeth Medica Ireland, Co. Kildare, Ireland) in study II and Minulet® (Wyeth Medica Ireland, Co. Kildare, Ireland) in studies III & IV were supplied by Wyeth.

GnRH-analog (Suprefact®, Hoechst, Switzerland) in study II, and Econ/30® pills (1 mg NET Ac and 30 μg EE2; Orion, Helsinki, Finland) and activated charcoal (Carbo medicinalis®; Leiras Oy, Turku, Finland) in studies III & IV were purchased as commercially available packages.

4 CONCOMITANT MEDICATION

Chronic use of steroids and antibiotics and the use of estrogens and progestogens other than study medication was prohibited throughout study I. In study II the volunteer women agreed not to use any medication apart from the study drugs. In studies III & IV women using any drug known to interfere with the pharmacokinetics of pill steroids were excluded from participation. Such medication included injected or implanted estrogens, progestogens or androgens within the previous 6 months, CYP-enzyme inhibitors or inducing agents, broad-spectrum antibiotics and other substances which could affect the enterohepatic cycling of steroids.

5 ULTRASONOGRAPHY

In the present study, to detect follicular growth, the two largest follicles in any ovary were scanned in two dimensions and for both follicles the mean of these two measures was taken. An intravaginal probe (6.5 MHz, Hitachi EUB-405E; Hitachi Medical Corporation, Tokyo, Japan) was used at the Family Planning Clinic of the Family Federation of Finland in all studies. In the other two clinics, in study I intravaginal probes of 6–7 MHz were used.

6 DETERMINATION OF OVULATION

During the treatment period of study I, a serum progesterone concentration exceeding 9.6 nmol/L plus follicle rupture was considered to represent ovulation, this being used in studies by the Population Council. In studies II–IV follicular growth beyond 13 mm plus its rupture, and serum progesterone concentrations of >5 nmol/L were used to indicate ovulation (Hoogland and Skouby, 1993), as shown in Table 2.

7 SAMPLE HANDLING AND HORMONE ASSAYS

In all studies blood samples were collected by antecubital phlebotomy. However, in study III, during the days when frequent samples were collected for determination of pharmacokinetic parameters, blood was drawn via an intravenous catheter fitted in a forearm. Blood was allowed to clot at room temperature and then centrifuged; serum was separated and stored at -20°C until assayed. The serum samples were all analyzed simultaneously at the end of the study, with all samples from the same subject and for the same hormone being analyzed in the same assay in order to avoid inter-assay variation. Details of the hormone assays used are given in Table 7.

Table 7 Characteristics of hormone assays

Analyte	Method	Intra-assay CV%	Inter-assay CV%	Practical detection limit	Laboratory	Study
Estradiol	RIA[1]	5.0-7.3	10.4-16.6	55 pmol/L	STRL	I, IV
	RIA[2]	4.3	7.9	75 pmol/L	NIJ, LEU	I
	IFMA[3]	3.8-10.0	3.6-9.7	50 pmol/L	FFF	II
Progesterone	IFMA[3]	3.3-7.3	2.7-10.1	0.8 nmol/L	STRL	I, IV
					FFF	II
	RIA[2]	4.1	9.1	1.3 nmol/L	NIJ, LEU	I
FSH	IFMA[3]	3.0-4.8	3.7-4.3	0.05 IU/L	STRL	I, IV
					FFF	II
	IRMA[4]	3.3-5.5	6.3-7.6	0.60 IU/L	NIJ, LEU	I
LH	IFMA[3]	3.7-4.7	2.4-7.5	0.05 IU/L	FFF	II
					STRL	IV
GEST	RIA[5]	5.5-7.3	7.6-11.7	0.3 nmol/L	STRL	III
NET	RIA[1]	6.3-9.9	8.5-17.2	0.3 nmol/L	STRL	III

[1]Modified according to Sufi *et al.*, 1991, [2]Modified according to Thomas *et al.*, 1977, [3]Delfia®, [4]Described in Kremer *et al.*, 1991,
[5]Modified according to Nieuweboer *et al.*, 1989
STRL=Steroid Research Laboratory, Institute of Biomedicine, University of Helsinki, Helsinki, Finland;
FFF = Family Federation of Finland, Helsinki, Finland;
NIJ = Reproductive Endocrinology Laboratory, University Hospital of Nijmegen, Nijmegen, The Netherlands;
LEU = University Hospital of Gasthuisberg, Leuven, Belgium.

8 STATISTICAL ANALYSIS

In study I the sample size estimate was made on the basis of the proportions of women who developed follicles with a diameter of 18 mm or greater. The study was designed to detect a difference between proportions of 25% and 60% in two treatment groups with 80% power, using 2-sided tests. Accordingly, 35 women were enrolled to ensure that 30 could be evaluated in each treatment group (i.e., 105 enrolled to ensure 90 for evaluation in total). Comparison between the MG, TG, and MD groups regarding the largest follicle and subject demographics was carried out by 1-way analysis of variance. Concentrations of FSH and estradiol were analyzed by analysis of variance for repeated measurements. Differences between means were compared pair-wise by linear contrast within the analysis of variance model.

In studies III and IV all the analyses were performed as 2-sided tests. For all continuous variables the assumption of normality was checked by examining the residuals. Analyses were carried out both with and without the extreme observations (outliers) if the assumptions of normality were not fulfilled. If the results of statistical tests were similar with and without the extreme observations, results with all observations are reported; otherwise results of both analyses are reported.

In study III, for $AUC_{0\text{-}24h}$, C_{max} and t_{max}, paired t-tests were used to analyze the difference between charcoal and control cycles for each pill. The difference from control cycle to charcoal cycle between the 2 pills was tested using analysis of variance for a cross-over design.

In study IV, for LH, FSH, estradiol and the diameter of the leading follicle, individual mean values from each period of one week (days 1–7, 8–14, 15–21 and 22–28) were used as outcome variables. Analysis of variance for repeated measurements was used to test the difference between the charcoal and control cycles, separately for each pill (NET Ac and GEST). The difference in the control cycle vs. the charcoal cycle between the two pills was tested using analysis of variance for a cross-over design with repeated measurements within periods.

All statistical analyses were carried out with SAS software (SAS Institute Inc., version 6.12, Gary, NC, USA). A p-value less than 0.05 was considered statistically significant.

9 RECORDING COMPLIANCE

Diaries were supplied in order to mark down pill intake, as well as the occurrence of spotting, bleeding and possible concomitant medication. In studies II–IV, exact times of pill taking were also recorded.

In order to decrease unintended pill omissions, in study II the pill was ingested, when scheduled, under an investigator's supervision on those days when the women came to the clinic for follow-up visits.

In studies III & IV the pill was ingested under the investigator's supervision during the most critical days, i.e. on days 13, 14 and 15 in both the control and charcoal treatment cycles during the use of both pills (NET Ac and GEST).

V RESULTS

1 RECOVERY OF THE PITUITARY-OVARIAN AXIS DURING AND AFTER THE PILL-FREE PERIOD

1.1 Extension of the pill-free period to ten days (Study I)

Mean serum estradiol concentrations during and after the standard 7-day pill-free period are illustrated in Figure 4a and those during and after the extended pill-free period in Figure 4b. The respective serum FSH levels are shown in Figures 4c and 4d.

In most women, serum FSH concentrations rose to levels normally seen in the late follicular phase as soon as the end of the first pill-free week (Figure 4c). This indicates rapid restoration of pituitary activity after stopping OCs. When the pill-free period was extended beyond 7 days, no further increase in serum FSH levels was seen. This is clearly illustrated in Figure 4d. In fact, despite three more days without pills, serum FSH concentrations started to decrease after the first 7 pill-free days, suggesting the possibility of a negative feedback effect to the excretion of endogenous estradiol.

In the comparison of three different OC preparations, namely monophasic GEST (MG), triphasic GEST (TG) and monophasic DSG (MD), the MG preparation seemed to suppress pituitary function most effectively during and after the standard 7-day pill-free interval (Figure 4c). The overall mean serum FSH concentrations were statistically significantly lower in the MG group than in the TG ($p=0.03$) and MD groups ($p=0.03$). During and after the extended pill-free period of 10 days (Figure 4d) no difference was seen between the two monophasic preparations, whereas the mean FSH levels associated with TG pills were higher than in women using the MG preparation ($p=0.02$) and those in the MD group, even though the latter difference was nonsignificant ($p=0.05$). However, after resuming the pills, pituitary function was rapidly suppressed again with all three treatments, both after the standard pill-free period of 7 days and after the extended pill-free period (Figures 4c and 4d).

After stopping OCs, serum estradiol concentrations started to increase, but considerable intersubject variation was seen, during and after the 7- and 10-day pill-free periods. This indicates great variability in both degree of ovarian activity as well as in timing of restoration of ovarian function after stopping pill intake. However, estradiol levels were consistently higher when the pill-free period was extended to 10 days (Figure 4b), compared with the standard pill cycle with a 7-day pill-free period (Figure 4a).

As shown in Figure 4a, the overall mean serum estradiol levels were higher in women using the MD preparation than in women in the other two groups, during and after the 7-day pill-free period (MD vs. MG p=0.01; MD vs. TG p=0.01). After the 7-day pill-free period, when the pills were restarted, mean serum estradiol levels decreased with all OC preparations, although this was more pronounced as regards pills containing GEST (Figure 4a). During and after the 10 pill-free days (Figure 4b), women in the MD group showed higher serum estradiol concentrations, compared with women using TG pills (p=0.01). Despite reintroduction of OCs, serum estradiol levels continued to increase in the MD group, whereas they remained virtually unchanged in women using MG or TG pills (Figure 4b). Subjects in the MG and TG groups did not differ from each other in either of the monitoring periods.

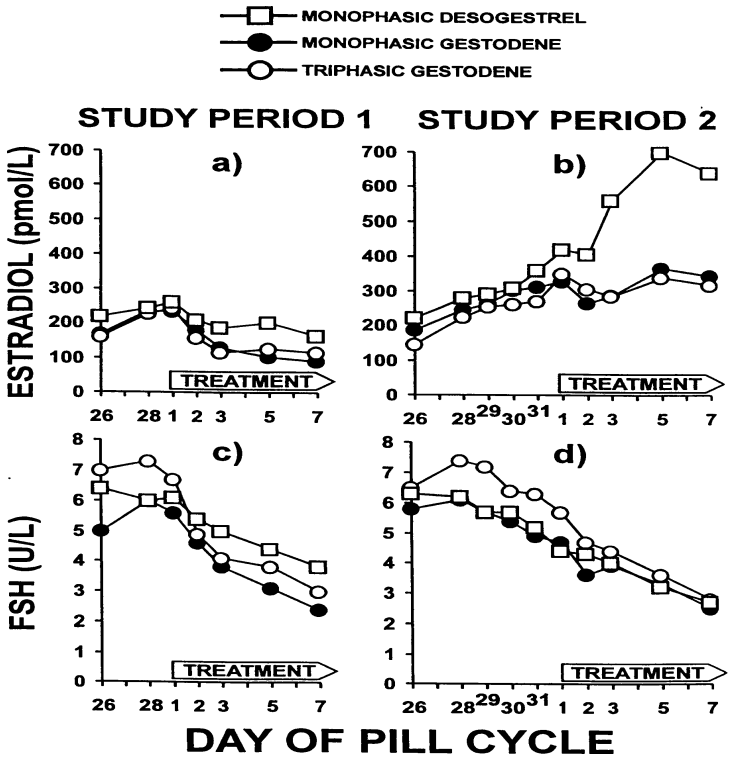

Figure 4 **Mean serum estradiol (a and b) and FSH (c and d) concentrations during and after the standard 7-day pill-free period (study period 1) and when the pill-free period was extended to 10 days (study period 2) in women using 1 of 3 OC preparations**

1.2 Extension of the pill-free period until the leading follicle had reached a diameter of 16 mm (Study II)

In 5 women using an OC preparation consisting of 75 µg of GEST and 20 µg of EE_2 (Harmonet®) the pituitary gland was well suppressed at the end of the regular OC cycle, as indicated by very low serum concentrations of FSH and LH on day 21 (Figures 6a and 6b, respectively). Consistently with the results from study I, serum FSH concentrations rose to the level normally seen in the late follicular phase of the cycle as early as the end of the normal 7-day pill-free period (data not shown). The mean concentration of serum LH had also clearly increased by pill-free day 7 but it continued to rise for a couple of days to reach its late follicular phase level. Figure 6c illustrates wide intersubject variation in the concentrations of serum estradiol.

2 FOLLICULAR GROWTH AND OVULATION

2.1 Extension of the pill-free period to ten days (Study I)

In study I no ovulation was seen in any of the three regimens, either during or after the standard 7-day pill-free period or when the pill-free period was extended by three days, as judged by serum progesterone levels of <9.6 nmol/L and by absence of rupture of the leading follicle.

However, during and after the 7-day pill-free period, 14% of the subjects showed a follicle greater than 13 mm. Follicles exceeded a diameter of 13 mm in 9%, 6% and 27% of the women in the MG, TG and MD groups, respectively, whereas only two women developed a follicle of 18 mm or greater after the regular pill-free period of 7 days. Both belonged in the MD group, representing 6.7% of women in that group.

When the pill-free period was extended to 10 days, in 53% of the women follicles grew beyond 13 mm, in 41% of MG users, 47% of TG users, and in 70% of MD users. A preovulatory follicle of >18 mm occurred in a total of 29% of the women after the 10-day pill-free period (in 24% of both MG and TG pill users, and in 40% of MD users).

There were three more women (1 in the TG group and 2 in the MD group) who showed follicular growth beyond 13 mm during or after the 7-day pill-free period. However, they were withdrawn from the study prior to when it was decided that women with a persisting follicle were allowed to continue in the study. Thus, these women were not included in the statistical analysis. One more woman was not included in the statistical analysis, as she already had a persisting follicle-like structure of 45 mm in diameter at the first monitoring.

As illustrated in Figure 5, follicles became largest in women using the MD preparation. The difference was statistically significant between the MG and MD groups ($p<0.022$) and between the TG and MD groups ($p<0.001$) during and after the 7-day pill-free period. After deliberate omission of 10 pills the mean

maximal follicle size in the MD group was statistically significantly greater than in the MG group (p=0.015).

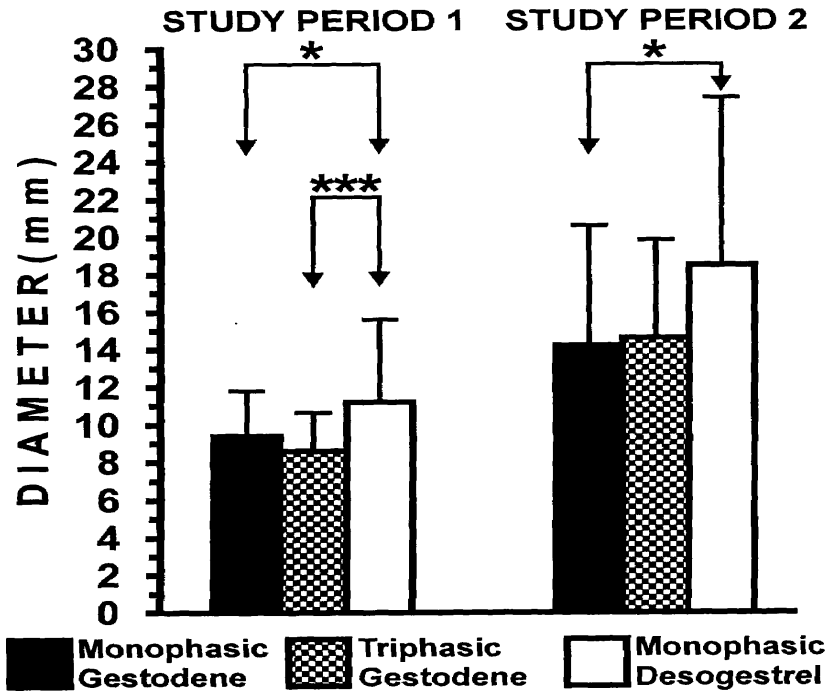

Figure 5 **Mean (±SD) maximal diameters of follicles during and after the 7-day pill-free period (study period 1) and the 10-day pill-free period (study period 2) in women using 1 of 3 combined oral contraceptive preparations (* p<0.05; *** p<0.001).**

2.2 Extension of the pill-free period until the leading follicle had reached a diameter of 16 mm (Study II)

When the pill-free period was deliberately extended until the leading follicle reached a diameter of 16 mm, four of five women ovulated. For four women it took 14 to 22 days to grow a follicle of 16 mm in diameter, after stopping the previous pill pack (day 21). The mean (±SD) serum FSH, LH, estradiol and

progesterone concentrations in the four ovulating women are shown in Figures 6a, b, c and d, respectively. As indicated in Figures 6a and 6b, in all women both FSH and LH surges occurred soon after the pills were restarted on day 1. The mean maximum serum LH concentration was 23.5 IU/L.

Individual maximum serum progesterone concentrations (Figure 6d) in the 4 ovulating women ranged between 10.7–23.0 nmol/L (mean 15.6 nmol/L).

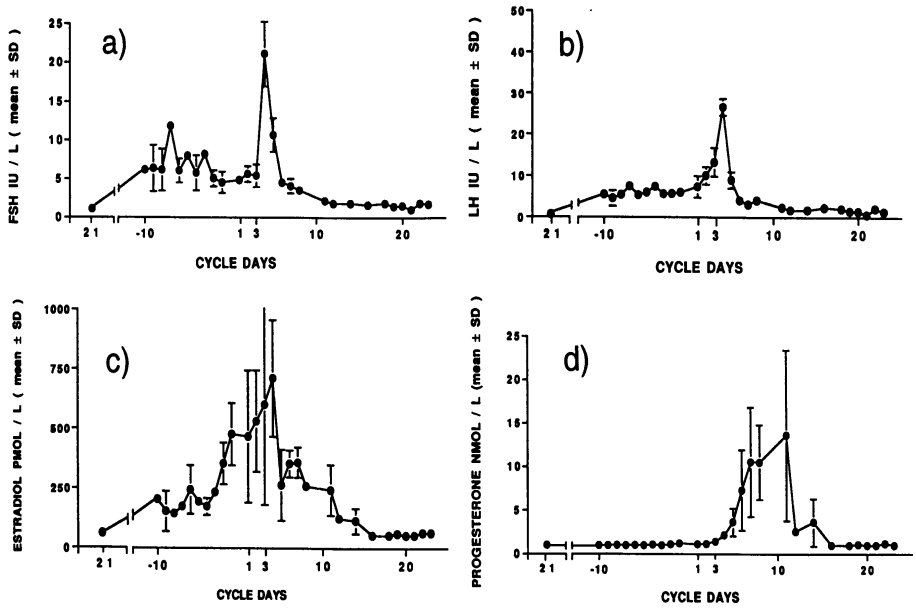

Figure 6 **Mean (±SD) serum concentrations of (a) FSH, (b) LH, (c) estradiol and (d) progesterone in four ovulating women in study II during the extended pill-free period, and in a subsequent cycle starting on day 1 (follicle diameter = 16 mm). Day 21 represents the last pill-taking day of the previous contraceptive cycle.**

An intravenous injection of GnRH analog was given on the third day (day 3) after reintroduction of the pills. In one woman an LH surge and rupture of a follicle took place just before GnRH administration (early morning of the same day), and in three ovulating women the peak concentration of serum LH was

measured within 24 hours after the intravenous bolus of GnRH. However, in these three women also, serum LH levels had started to increase before the GnRH bolus, as indicated by a 30% increase in the mean concentration of three consecutive LH samples in the midfollicular phase of the cycle (Yen, 1991). This suggests spontaneous onset of a preovulatory LH surge (Figure 6b).

The dominant follicles of four ovulating women ruptured at a mean size of 20.7 mm. Ovulation occurred 2 to 7 days after reintroduction of OCs, i.e. 0 to 5 days after the i.v. GnRH bolus. Development of the leading follicle in each ovulating woman is shown in Figure 7.

Although the criteria of ovulation (Hoogland and Skouby, 1993) were fulfilled in study II, ovulation did not seem to be completely 'normal' in any of the four women. In one woman a follicle ruptured as late as 4 days after the LH peak and 5 days after the intravenous bolus of GnRH analog. The maximum serum progesterone concentration in this woman was 15.0 nmol/L. Furthermore, in all four ovulating women the overall serum progesterone levels remained well below those seen in normal ovulatory cycles, and no preovulatory rise in progesterone levels was seen in any woman (Figure 6d).

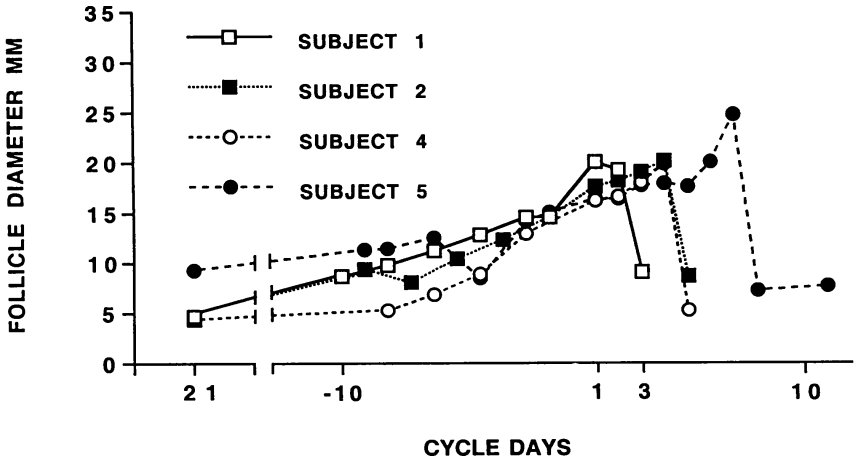

Figure 7 **Follicle diameter in four ovulating women. Day 1 is the first day of the new pill pack. GnRH analog was given on Day 3.**

3 LUTEINIZED UNRUPTURED FOLLICLES

In study I, serum progesterone levels remained under 9.0 nmol/L in all cases, except for one woman in the MG group who showed signs of a luteinized unruptured follicle (LUF). Her serum progesterone level had increased sharply to 21.9 nmol/L on treatment day 3, after the extended pill-free period. On the same day a follicle of 12 mm in diameter was measured, and it grew to a size of 21 mm by pill cycle day 7. By then, the serum progesterone concentration had dropped well below the ovulatory level (2.2 nmol/L), and thereafter the follicle diminished gradually without rupturing. The highest measured estradiol level was only 319 pmol/L throughout monitoring.

In study II one woman had signs consistent with a luteinized unruptured follicle. Also with her, steep increases in the serum concentrations of both FSH and LH were seen after the reintroduction of an OC. A dominant follicle reached a diameter of 16 mm as late as after 26 pill-free days, and it was accompanied by a normal preovulatory estradiol level of 920 pmol/L. The highest progesterone concentration throughout the observation period was only 5.8 nmol/L and the dominant follicle diminished in size without rupturing, after it had reached a diameter of 31.2 mm.

4 INTERRUPTION OF ENTEROHEPATIC RECIRCULATION OF NORETHISTERONE AND GESTODENE BY CHARCOAL TREATMENT

4.1 Bioavailability of norethisterone and gestodene (Study III)

The bioavailability of NET and GEST was evaluated in 11 women using either a preparation containing 75 µg GEST and 30 µg EE_2 (GEST pills) or 1 mg NET Ac and 30 µg EE_2 (NET Ac pills) for four months and thereafter the other treatment for 4 subsequent months. More specifically, C_{max}, t_{max} and $AUC_{0\text{-}24h}$ were measured for NET and GEST after repeated ingestion of activated charcoal in mid-cycle, and during the regular cycle serving as a control.

No statistically significant differences were seen in any of the measured parameters between the control and charcoal cycles, with either pill or between the two pills (GEST pills and NET Ac pills). The mean $AUC_{0\text{-}24h}$ ±SD for GEST during the control cycle was 547 ± 384 nmol×h/L compared with 544 ± 292 nmol×h/L in the charcoal cycle (p=0.95). During the use of NET Ac pills the respective values for NET in the control and charcoal cycles were 604 ± 261 nmol×h/L and 540 ± 244 nmol×h/L (p=0.15).

Mean serum GEST and NET concentrations during 24 hours are shown in Figures 8 and 9, respectively. The mean (±SD) C_{max} values of GEST in the control and charcoal cycles were comparable, 42.4 ± 20.6 nmol/L and 40.2 ± 18.0 nmol/L, respectively (p=0.25). The maximum concentration of GEST was reached in both the control and charcoal cycles within 110 min (mean t_{max} ± SD was 82 ± 34 min in the control GEST pill cycle and 95 ± 35 min in the charcoal GEST pill cycle). Neither was a difference seen in the mean C_{max} values of NET between cycles. The mean (± SD) maximum concentration was 75.4 ± 29.0 nmol/L in the control cycle and 69.7 ± 28.0 nmol/L in the charcoal cycle (p=0.32). T_{max} (mean ± SD) for NET was 124 ± 39 min in the control cycle (range 60–120 min) and 124 ± 60 min in the charcoal cycle (range 60–180 min). When the difference from the control cycle to the charcoal cycle between the two pills was tested, utilizing $AUC_{0\text{-}24h}$ and C_{max} values calculated in the control and charcoal cycles of each pill treatment, no difference between GEST and NET was seen (for $AUC_{0\text{-}24h}$ p=0.29 and for C_{max} p=0.42). In other words, activated charcoal did not have a different effect on the bioavailability of the two progestogens.

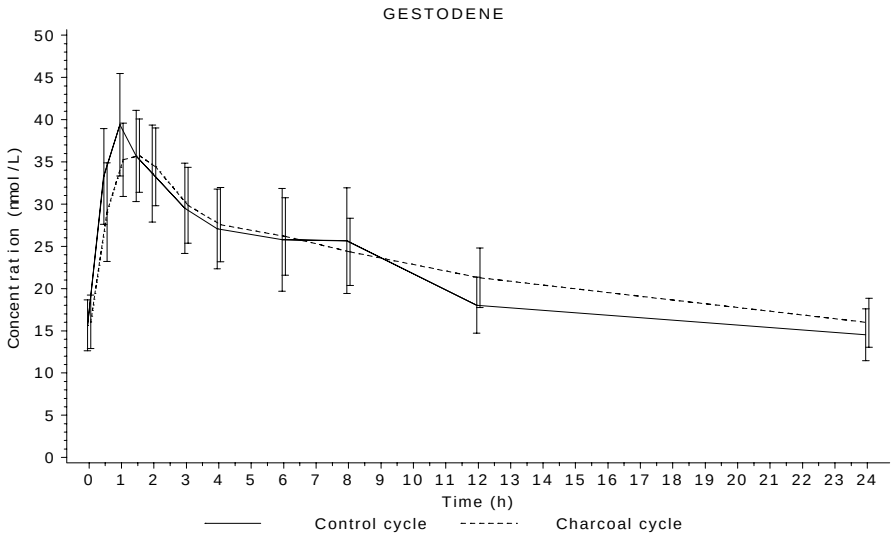

Figure 8 **24-h mean (± SD) serum gestodene concentrations during the regular contraceptive cycle and during the cycle with charcoal treatment in women using a monophasic gestodene preparation (75 µg GEST and 30 µg EE_2).**

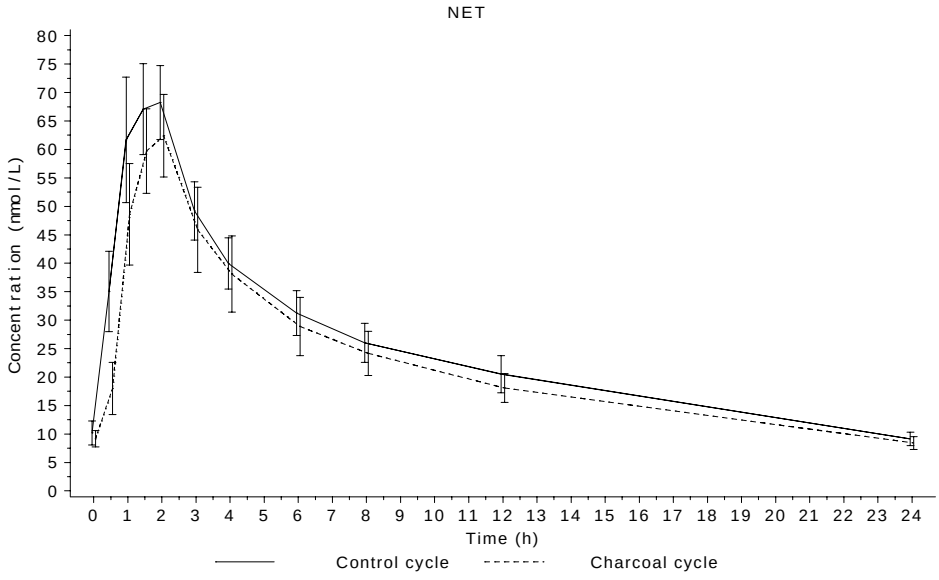

Figure 9 **24-h mean (± SD) norethisterone concentrations during the regular contraceptive cycle and during the cycle with charcoal treatment in women using a monophasic norethisterone acetate preparation (1 mg NET Ac and 30 µg EE_2)**

4.2 Charcoal treatment and risk of ovulation (Study IV)

No ovulation was detected in 11 women under either OC treatment (GEST or NET Ac pills), whether charcoal was given or not. Serum progesterone concentrations in all women remained well under the ovulatory level (<3 nmol/L, data not shown). Follicles also remained small in all cases but two. One subject developed a preovulatory sized follicle during both the control and charcoal treatment cycle of NET Ac pill use, whereas in the other subject follicles grew beyond 13 mm in diameter during the control and charcoal treatment cycles of both pill regimens. A clear increase in serum estradiol concentrations was seen in both women, but no rupture of follicles was detected.

Activated charcoal treatment did not seem to affect pituitary-ovarian function associated with either pill treatment, as evaluated by measurements of LH, FSH and estradiol concentrations and the diameter of the leading follicle. Neither was a different effect seen between the two pills.

With either pill, when control and charcoal treatment cycles were compared, no statistically significant differences were seen in the mean serum concentrations of LH (GEST p=0.58; NET p=0.33), FSH (GEST p=0.40; NET p=0.06) or estradiol (GEST p=0.36; NET p=0.37), or in the mean diameter of the leading follicle (GEST p=0.57; NET p=0.27). When each week (days 1–7 = week 1, days 8–14 = week 2, days 15–21 = week 3 and days 22–28 = week 4) of the control and charcoal treatment cycles was compared, with either pill, no statistically significant differences were detected in any of the measurements.

Differences in the mean serum concentrations of LH, FSH and estradiol, and the mean diameter of the leading follicle from the control to the charcoal treatment cycles were compared between GEST and NET Ac pill treatments on a weekly basis. No statistically significant differences appeared in this analysis with regard to any of the measured parameters (p-values for LH, FSH, estradiol and follicle diameter were 0.33, 0.09, 0.69 and 0.76, respectively).

In addition, the time-effect was not statistically significantly different between the two pill treatments as regards LH (p=0.13), estradiol (p=0.23), or the mean diameter of the leading follicle (p=0.92). In the cross-over analysis FSH concentrations showed a significant difference in the treatment vs. time interaction (p=0.02), although not when the highest FSH value of 14.5 IU/l in one subject was excluded (p=0.05).

5 COMPLIANCE IN PILL TAKING

In addition to the intended pill omissions for 3 consecutive days in study I, four subjects accidentally forgot 1 pill and 1 subject forgot 2 pills within 7 days after the 10-day pill-free period. Moreover, two subjects omitted one pill during the first pill-taking week after the standard 7-day pill-free period in study I. These subjects were, however, included in the statistical analysis, as these further omissions did not affect the subjects' individual hormone profiles, overall follicular growth or increase in serum estradiol levels.

The most critical parts of studies II–IV were secure with regard to pill taking. In study II no additional pill omissions were reported. In studies III and IV one subject missed one pill (pill number 17 in the first NET Ac pill cycle), and another woman reported four 24-hour late pills (one in the second NET Ac pill cycle, 2 pills in the second GEST pill cycle and one, pill number 18, in the third GEST cycle). Moreover, six women reported 2- to 10-hour late pills a total of 32 times. In none of these cases was the timing of missed or late pills critical in terms of the measurements performed in studies II–IV.

6 CONCOMITANT MEDICATION

Five women used either penicillin or tetracycline or their derivatives for 7 to 10 days in study I. In two women using these antibiotics during the control pill cycle,

follicular development and elevated serum estradiol levels were demonstrated after the extended pill-free period. In these two cases it is unlikely that the antibiotic would have influenced ovarian activity over one completely suppressed cycle. Enlarged follicles were detected after the extended pill-free period in one woman who had used a penicillin derivative during and after the 7-day pill-free interval. Even though unlikely, we cannot rule out the possibility that the follicular activity seen was influenced by concurrent medication. No follicular activity was detected in two of the 5 women.

In studies II–IV none of the women reported the use of any concomitant treatment known to interfere with steroid metabolism.

VI DISCUSSION

The failure rate of the combined oral contraceptive method, when used correctly, is considered to be very low, the Pearl index being only 0.1 pregnancies per 100 woman-years (Hatcher *et al.*, 1994). Under the circumstances of user failure, for various reasons, the Pearl index for OCs may increase to 0.5–5 per 100 woman-years, with great variability between different populations (Potter, 1996). Failure is generally thought to be associated with missed or late pills, gastrointestinal upsets or interaction with other drugs. Therefore, in the present study, conditions simulating these circumstances were created. However, only 4 women ovulated, all after deliberate omission of at least 14 consecutive pills and when pills were restarted at the stage when the diameter of a dominant follicle had reached 16 mm.

Ovulation and possible pregnancy during pill intake can only occur when residual ovarian activity appears and further follicular maturation and a mid-cycle LH surge is allowed. Besides ovulation prevention, OCs also have a number of other contraceptive effects which may or may not persist in the case of escape ovulation. In a few clinical studies, in which cervical mucus has also been investigated in connection with deliberate pill omission, the mucus has remained 'hostile' despite measurable ovarian activity (Chowdhury *et al.*, 1980; Hamilton and Hoogland, 1989; Killick *et al.*, 1990; Morris *et al.*, 1979; Spona *et al.*, 1993). However, adjunctive actions such as this may be least effective at the end of the pill-free period, when restoration of ovarian activity is also anticipated (Guillebaud, 1981). Moreover, in cases of accidental pregnancy in women using OCs, these adjunctive mechanisms of action are clearly insufficient. Hence, to ensure efficacy of an OC, inhibition of pituitary FSH, which promotes follicular growth and maturation, should be as complete as possible.

1 OC REGIMENS AND THEIR POTENCY TO INHIBIT OVULATION

It is well known that the pituitary is not completely suppressed even with old preparations containing 50 μg of EE_2 (Elstein *et al.*, 1974). Hence, it is surprising that contraceptive efficacy does not seem to be appreciably compromised during regular use of OCs containing only 20 μg of EE_2, when combined with traditional doses of gestodene (75 μg) or desogestrel (150 μg) and when the pills are taken regularly (Crosignani *et al.*, 1996; Endrikat *et al*., 1995; Endrikat *et al*., 1997; Endrikat *et al*., 2001; Fitzgerald *et al.*, 1994; Rosenberg *et al*., 1999; Spona *et al.*, 1996a; van Heusden and Fauser, 1999). However, little is known about ovulation inhibition potency of low-dose and ultra low-dose regimens, when compliance is poor.

In this study a combination of 150 μg of desogestrel and 20 μg of EE_2 resulted in less ovarian suppression during and after the 10-day pill-free period, compared with gestodene preparations with at least 30 μg EE_2 per day. Whether this is due to the lower dose of EE_2, or a different progestogen component, or both, cannot be proven, as no control was used as regards the same progestogen component, and dose. From earlier studies there is no agreement on the relative importance of an EE_2 dose compared with a progestogen component in inhibition of residual ovarian activity (Fitzgerald *et al.*, 1994; Mall-Haefeli, 1991; Römmler *et al.*, 1985; van Heusden and Frauser, 1999).

Because there is a great deal of evidence indicating that OCs are reliable and safe, new research is concentrated on ultra-low dose regimens, to further minimize unfavorable effects, rather than investigation of underlying mechanisms of action, such as regulation of the hypothalamus-pituitary-ovarian axis. It is believed that in OCs progestogen prevents the mid-cycle LH surge, whereas pituitary inhibition of FSH release is probably caused by both EE_2 and progestogen, the overall effect being synergistic (Mishell *et al.*, 1977, Römmler *et al.*, 1985). Hence, interaction between synthetic OC steroids and pituitary gonadotropins may be more complex than might be interpreted from the sum of progestogenic and estrogenic components of the regimen.

Newer progestogens not only differ greatly in their structure, but they show considerable differences in their pharmacokinetics and dose-effect responses (Kuhnz and Gieschen, 1998). Furthermore, EE_2 may express its inhibitory effect at the pituitary only within a certain dose range. Thus, a regimen with a biologically potent progestogen, such as gestodene, may suppress pituitary-ovarian function effectively when combined with only 15 µg EE_2 (Gestodene Study Group 322, 1999), but this may not apply to other progestogens with lesser potency to inhibit ovulation (Kuhl, 1996; Römmler *et al.*, 1985).

As follicular maturation may be initiated during the 7-day pill-free period, and in triphasic preparations the progestogen dose is lowest during the first phase of the cycle, it has been speculated that triphasic OCs may result in less complete pituitary-ovarian suppression than monophasic regimens. In the present study a similar degree of ovarian suppression was achieved with mono- and triphasic preparations, both containing gestodene. This is in contrast to results obtained mainly from some case-reports and uncontrolled studies (Caillouette and Koehler, 1987; Kovacs *et al.*, 1989; Römmler *et al.*, 1985; Smith *et al.*, 1986; Van der Vange *et al.*, 1985), and in line with results from more strictly controlled studies carried out in conditions of regular use of OCs (Crosignani *et al.*, 1996; Gaspard *et al.*, 1984; Grimes *et al.*, 1994; Holt *et al.*, 1992*;* Killick *et al.*, 1990; Lete and Morales 1997; Spona *et al.*, 1993*;* Young *et al.*, 1992).

2 ENTEROHEPATIC RECIRCULATION OF OC STEROIDS

A substantial number of case-reports have been published on pregnancies occurring during the concomitant use of OCs and broad-spectrum antibiotics (Back *et al.*, 1988; Bacon and Shenfield, 1980; Bainton, 1986; DeSano and Hurley, 1982). Hence, instructions for women on OCs persistently include a warning regarding concurrent therapy with broad-spectrum antibiotics, although scientific data from controlled studies do not support an interaction (Back *et al.*, 1982b; Friedman *et al.*, 1980; Joshi *et al.*, 1980b). Broad-spectrum antibiotics have been shown to interfere with the normal intestinal bacteria participating in

hydrolysis of steroid conjugates. This may interrupt enterohepatic recirculation of contraceptive steroids by preventing reabsorption of the steroids. Whether this is relevant with regard to progestogens or even with EE_2, has still not been convincingly proven. Even if it occurs, its clinical importance may be negligible (Back *et al.*, 1990), and certainly anecdotal case-reports do not justify any causal relationship. We therefore tested whether repeated ingestion of activated charcoal on three consecutive days would affect the bioavailability of two progestogens, norethisterone and gestodene, and whether it impaired pituitary-ovarian suppression under two different OC regimens. The circumstances created also simulated conditions where enterohepatic recirculation of contraceptive steroids would be affected by treatment with broad-spectrum antibiotics, or by gastroenteritis. Even though pill omissions at mid-cycle are anticipated to confer less risk of contraceptive failure than extended pill-free intervals (Guillebaud, 1987; Molloy *et al.*, 1985), charcoal was administered at mid-cycle, as the risk of ovulation had been already elucidated during and after the pill-free period.

As activated charcoal did not alter any of the pharmacokinetic parameters, compared with non-charcoal treatment cycles, it can be concluded that enterohepatic recirculation does not occur with these two progestogens to such an extent that it would be of any clinical importance. Although two women showed follicular maturation and growth beyond a diameter of 13 mm, it is unlikely that charcoal would have had any influence, as in both women follicles developed in both the charcoal and control cycles. No ovulation occurred in connection with either pill, regardless of the administration of activated charcoal.

Even though the study set-up did not reflect exactly the situation in gastroenteritis, which involves inflammation of the intestinal wall (Adlercreutz *et al*., 1979), it is considered that disturbance of enterohepatic circulation would have been demonstrated if it is of importance. This is because the dosage of activated charcoal used in the present study has been previously shown to be effective in a study on mifepristone (Heikinheimo *et al.*, 1989) and it was double the dose used in the treatment of diarrhea. Nevertheless, there may be a group of women, slow metabolizers, in whom there is no hydroxylation and who are

thus more dependent on enterohepatic recirculation of steroids. It is most likely, however, that many of the pill failures in women using OCs and broad-spectrum antibiotics concurrently are due to noncompliance in pill taking.

3 MISSING PILLS – AN INHERENT PROBLEM WITH OCS

Noncompliance as regards missed or late pills is an inherent problem with OCs in both clinical studies that aim at elucidating the problem as well as in clinical practice. In a large proportion of women in the present study, additional missed or late pills could not be reliably recorded and eliminated. However, the risk of drawing the wrong conclusions concerning ovulation and pill omission is minimal, because in the particular study in which all cases of ovulation occurred, pill taking was closely monitored.

In an earlier study on 'reliable pill takers', more than a third of the women who experienced an accidental pregnancy during OC use did not report any known predisposing factor (Sparrow, 1998). It was suggested that these cases were due to method failure, whilst two thirds of the pregnancies could have been associated with predisposing factors other than noncompliance in pill taking.

Even though women on pills occasionally appear to become pregnant without any obvious underlying reason, it is now known that missing pills is much more common than is generally recognized. It has been shown only recently, by using a reliable method involving a microchip-augmented pill dispenser, that more than 30% of women miss three or more pills during their first two treatment cycles, and during the third cycle more than 50% of users miss that number (Potter *et al.*, 1996). Discrepancy between this outcome and results obtained in interview studies indicates that missed pills are underreported to a great extent. The poorest pill takers are often also those who can least well recall the number and timing of the missed pills (Potter, 1996).

4 THE PILL-FREE PERIOD AND THE RISK OF ESCAPE OVULATION

An OC regimen of 21 pills, followed by 7 pill-free days, was initially introduced by doctor Gregory Pincus to mimic a regular menstrual cycle of 28 days (Fraser and Jansen, 1983). A monthly cycle for the first OC was, however, chosen arbitrarily to make the pill more acceptable at a time when oral contraception was still a novel concept. It was not to become mandatory, but merely to be developed in the next stage to allow a longer period of active medication (Rutter *et al.*, 1988). Although an OC regimen of 21 days on and 7 off offers probable benefits, such as a lesser total quantity of OC steroids during the 4-week period and, through withdrawal bleeding, a monthly check-up of not being pregnant, it also has a great disadvantage. The pill-free interval may not only allow restoration of pituitary-ovarian activity, and in many women recruitment of follicles, one of which will be later selected as a dominant follicle, but it may also lead to further pill omissions, as the pill-taking routine must be re-established after each period of cessation of the pills.

Full pituitary recovery has been shown to take place as early as during the standard 7-day pill-free period, even with OCs containing 30 μg of EE_2 (Killick *et al.*, 1987), and the return of full contraceptive efficacy takes 7 days (Guillebaud, 1987). Thus, the first week of pill-taking is critical for the contraceptive efficacy of OCs. Furthermore, it is often not realized that the pill-free period will also become extended if the last pills of the previous cycle are forgotten and the next cycle is started correctly (Guillebaud, 1987).

The growth of follicles has been detected in over 50% of normal OC cycles (Spona *et al*., 1996a; Van der Vange *et al.*, 1985). Specifically, follicles greater than 10 mm in diameter, the size considered a prerequisite for their further development, have been observed on pill-free day 7 in 18 to 27% of women (Tayob *et al.*, 1990; van Heusden and Frauser, 1999), and in 9 to 37% of women any time of a regular cycle (Crosignani *et al*., 1996; Fitzgerald *et al*., 1994; Hamilton and Hoogland, 1989). Preovulatory follicles of >18 mm in diameter have been found in roughly every third cycle, after the standard 7-day

pill-free period (Van der Vange *et al.*, 1985). In accordance with these findings, in the present study 14% of women developed a follicle of 13 mm or larger during or after the 7-day pill-free period. When the pill-free period was extended to 10 days, 53% of the women had a follicle of 13 mm or greater, and follicular growth to >18 mm occurred in almost one third of the women.

Even though follicles were ready for ovulation and the pituitary had already recovered during the first 7 pill-free days, ovulation did not occur if the pill-free period was extended to 10 days. Hence, the growing follicles seem to lose their ability to ovulate or they do not receive an adequate signal for ovulation, if the pills are started early enough in the growing process. On the other hand, after 14 pill-free days or more and if preovulatory follicles had reached a diameter of 16 mm, ovulation could not be prevented by restarting the pills. An injection of GnRH analog was given to detect susceptibility of the follicles to ovulate, but in all 5 women the LH surge had taken place before GnRH injection. This suggests a spontaneous start of ovulation in four women who also showed rupture of the leading follicle.

In an earlier study HCG was administered at the stage when the leading follicle was 18 mm in diameter (Killick, 1989). It was concluded that ovulation could be induced by HCG, indicating the readiness of preovulatory follicles of that size to ovulate if pill omission took place. In that study, follicles were allowed to grow to 12 mm before resuming OCs. However, in that study also, spontaneous ovulation cannot be excluded, as serum LH concentrations were not measured and serum progesterone concentrations had started to increase before HCG injection. In another study, 6 of 10 women ovulated during a cycle starting after 14 days on placebo pills (Letterie, 1998). A follicle diameter of 15 to 20 mm was reached by the time OC pills were restarted. Consistently with results from the present study, the follicles ruptured at a mean size of 21 mm.

There appears to be a continuing tendency to further reduce OC steroid doses by searching for new progestogens and combinations of progestogens and an estrogenic component, whilst sticking to the original idea of having hormone-free

days every month. A regimen of 24 active pills with lower steroid doses (60 µg gestodene and 15 µg EE_2) allowing a shortened pill-free period of 4 days, has been introduced only recently. The contraceptive efficacy of this regimen has been shown to be comparable to that of a 21-day regimen with conventional doses of OC steroids (Gestodene Study Group 322, 1999; Spona *et al.*, 1996a; Sullivan *et al*., 1999). However, shortening the pill-free interval does not solve the problem of compliance. Continuous use of OCs for at least three months has been proposed previously in order to avoid withdrawal headache and to allow bleed-free treatment, but not to safeguard contraceptive efficacy. Replacing hormone-free days with placebo pills is an attempt to benefit women with poor compliance, but it does not offer the above-mentioned benefits.

5 WHAT ADVICE CAN WE GIVE?

There is no longer a reason to utilize the initial idea of a 28-day regimen including 7 hormone-free days, especially as the steroid doses are only fractions of the ones introduced 40 years ago. Oral contraceptives could be used for a minimum of three pill packs in a row to minimize 'additional' pill omissions on top of the 7 permitted ones. However, as long as traditional OC regimens of 21 active days with 7 pill-free days are used, and as it is not yet possible to predict those women in whom pill failure can most readily occur, the present data allow the following considerations:

The risk of ovulation is considered small if less than four pills of one pill packet are omitted, provided that they do not lengthen the pill-free period, they do not occur successively and only one of the missed pills is during the first 7 days of pill intake.

If the first three pills of a new packet, or the last three pills of the previous packet have been omitted, thereby leading to an extended pill-free period of ten days, ovulation is unlikely to occur if no more pills are missed during the first pill-taking week. However, a woman should use a barrier method for one week, as it is

believed that after the pill-free interval, restoration of contraceptive efficacy occurs during the next seven days of pill taking. When the last pills of the previous pack have been omitted, and the seven-day pill-free period is not yet exceeded, a new pill pack is recommended to start without a hormone-free period.

In the present study five women reported additional, ‘unscheduled’ pill omissions within seven days after the 10-day pill-free interval. However, in these particular cases contraceptive efficacy was not impaired, as these women did not show large follicles at the time of additional pill omission. There is no published data on the effect of cessation of pills during those seven days in circumstances where a preovulatory follicle exists. Neither was this investigated in the present study, as it would have required complicated mathematical models to exclude confounding factors. However, this situation is expected to be of the highest risk of escape ovulation. Therefore, if pills are omitted during the first 7 days of pill taking, preceded by a 10-day pill-free period, a hormonal emergency contraception should be taken and a barrier method used for one week.

It is almost impossible to determine the minimum total number and exact time of missed pills, which would confer a significant risk of ovulation. Hence, it is safest to use a barrier method until the start of a next pill pack, if a total of four pills or more have been omitted at any time of the pill cycle. Practical advice in cases of pill omission is shown in Table 8.

Table 8 Pill omissions, risk of ovulation and use of another method of contraception

Number of missed pills and preconditions	Risk of ovulation	Use of another method
≤3 non-consecutive pills, provided that: • They do not include the first or the last pill • Only one pill omitted during the first pill-taking week	Very small	Not needed
The last pills of the previous pill pack Omitted	Very small if the pill-free interval does not exceed 7 days	A new pill pack should be started immediately
Pill-free period lengthened to a maximum of 10 days with *no* additional pill omissions during the first 7 days of a new pack	Small	Barrier method for the first week of a new pill pack
Pill-free period lengthened to a maximum of 10 days with additional pill omissions during the first 7 days of a new pack	Considerable	Barrier method for the first week of a new pill pack and a hormonal emergency contraception
≥4 pills at any time of a pill cycle	Unpredictable	Barrier method until the start of a new pill pack

VII CONCLUSIONS

The overall results of this study indicate a high safety margin for contraceptive efficacy with OC regimens of 21 active pills and 7 days off, when 20 μg of EE_2 or more is combined with 75 μg of gestodene or 150 μg of desogestrel.

Follicles became most developed in the regimen consisting of 20 μg EE_2 and 150 μg desogestrel. Nevertheless, if the pill-free period is restricted to ten days and no additional pill omissions occur during the next week, the risk of escape ovulation remains small when 20 μg of EE_2 or more is combined with traditional doses of either gestodene or desogestrel. Furthermore, both triphasic and monophasic gestodene preparations show a similar degree of pituitary-ovarian suppression when at least 30 μg of EE_2 is taken daily. On the other hand, a pill-free period of 14 days or more, accompanied by a preovulatory sized follicle of 16 mm or more in diameter, leads ultimately to ovulation in most women. At this stage ovulation can no longer be prevented by restarting pills.

The results of the present study did not indicate that enterohepatic recycling of progestogens is of any clinical significance. Thus, the present data do not support the current instructions to patients insisting on the use of additional contraceptives in the case of concurrent use of broad-spectrum antibiotics and OCs. Moreover, although not popular among younger women using OCs, charcoal treatment may be used for the treatment of diarrhea, when administered 3 hours or more after pill intake and at least 12 hours before the next pill.

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